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THE EFFECT OF ZOOPLANKTON ON THE SURVIVAL OF *ESCHERICHIA COLI* IN MICROCOSMS (SDI)

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THE EFFECT OF ZOOPLANKTON ON THE SURVIVAL OF *ESCHERICHIA COLI* IN MICROCOSMS (SDI)

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DEDICATION

To my mother and father

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RÉSUMÉ

Escherichia coli, une des bactéries indicatrices fécales les plus couramment utilisées, joue un rôle clé dans le suivi et l'évaluation de la qualité de l'eau. Comprendre son devenir en tenant compte de divers facteurs environnementaux (ex. prédation par le zooplancton) est essentiel pour évaluer la qualité microbiologique de l'eau et de protéger le consommateur. De nombreuses études ont été menées sur les effets de la température, de l'irradiation solaire ou des nutriments. Quelques données limitées démontrent l'effet du broutage par le protozooplancton (nanoflagellés et ciliés hétérotrophes) sur le devenir d'*E. coli* dans l'eau. Cependant, la capacité du métazooplancton à influencer ou à contrôler l'indicateur fécal dans l'eau reste encore peu comprise, malgré l'omniprésence de cette communauté planctonique dans nos ressources aquatiques.

Dans cette étude, nous étudions l'impact du métazooplancton sur la survie d'*E. coli* dans l'eau. Une première série d'expériences a été menée en laboratoire afin d'étudier comment le métazooplancton, et en particulier le cladocère *Daphnia*, pouvait éliminer *E. coli* de l'eau dans diverses conditions en eau synthétique et en eau de lac. Ensuite, nos expériences visaient à comparer la pression de prédation de plusieurs représentants du métazooplancton sur *E. coli* dans de l'eau de surface. Enfin, les travaux se sont penchés sur l'étude de la variation saisonnière du taux de prédation (ou taux de broutage) sur *E. coli* par des communautés zooplanctoniques indigènes (à la fois méta- et protozooplancton) dans de l'eau de lac.

En microcosmes d'eau synthétique, l'espèce modèle *D. pulex* (32 ind.L^{-1}) ingère et induit un effet significatif sur la survie d'*E. coli*. Cependant, en réduisant d'un facteur 1000 la concentration initiale en *E. coli* dans l'eau, les taux d'abbattement diminent de 1.65 j^{-1} à 0.62 j^{-1}, témoignant d'une plus faible probabilité de rencontre entre *Daphnia* et *E. coli*. En eau de lac, l'influence de *Daphnia* sur les taux d'abattement d'*E. coli*. Augmente avec la densité de population du cladocère, et atteint près de 0.47 j^{-1} en présence de 65 ind.L^{-1}. Il est intéressant de noter également qu'un part majeur de la diminution des concentrations en *E. coli* est attribuable à des facteurs liés à la matrice - probablement par la présence d'autres communautés bactérivores et/ou par des effets de compétition bactérienne.

Dans une matrice constituée d'un mélange d'eaux usées et d'eaux de surface pour simuler un évènement de contamination fécale en présence de métazooplancton en densités de population

représentatives pour les eaux de surface, *D. magna* (36 ind. L^{-1}) semble exercer l'impact le plus important sur *E. coli* avec un taux d'abattement moyen de 2,33 j^{-1}. À densités de populations égales (36 ind. L^{-1}), *D. pulex* induit des taux d'abattement d'*E. coli* compris entre 0,99 et 0,62 j^{-1}. Le rotifère *Brachionus calyciflorus* possède quant à lui la plus faible pression de prédation sur *E. coli* avec un taux moyen ne dépassant pas 0,21 j^{-1}, cela malgré une densité de population plus importante (500 ind.L^{-1}). La raison la plus probable de leur faible impact sur *E. coli* semble provenir de leur taille plus réduite et par conséquent de vitesses de filtration nettement plus faibles que celles de *Daphnia*.

Enfin, nous mettons en évidence une certaine variabilité saisonnière du taux de broutage des communautés zooplanctoniques d'eau douce sur *E. coli*. Cependant, les raisons de ces variations semblent complexes à appréhender et nécessiteraient davantage de données. Des facteurs associés non seulement à la composition et l'abondance relatives des groupes de zooplancton mais également la température semble influencer la prédation sur *E. coli* dans les eaux de surface.

En conclusion, le rôle potentiellement épurateur de *Daphnia* se confirme pour *E. coli* dans l'eau. Cependant, il est important d'être conscient que les taux d'abattement d'*E. coli* dépendent de multiples facteurs, dont certains comme la ratio prédateur-proie, la taille du zooplancton (et leur taux de filtration) ainsi que le type de matrice et de ses composantes biotiques, ont été mis en évidence durant ce travail. Les résultats offrent une vision plus complète de l'effet du zooplancton sur *E. coli* dans l'eau et aident à expliquer les différences qui peuvent être observées à différents moments de l'année dans les milieux aquatiques naturels. Le type de données acquises au cours de ce travail devraient également permettre d'améliorer les modèles actuels sur le devenir et le transport d'*E. coli* dans l'eau.

ABSTRACT

Escherichia coli is one of the most commonly used fecal indicator bacteria (FIB), and thereby plays a key role in water quality monitoring and assessment. Understanding its fate under a variety of environmental factors (ex. predation by bacterivorous zooplankton) is essential for the assessment of water quality to ensure public health. Much research has been done on the effects of temperature, sunlight irradiation, or nutrient scarcity. Limited knowledge has been generated on the effect of protozooplankton grazing (heterotrophic nanoflagellates and ciliates) on the fate of *E. coli* in water. In contrast, the capability of metazooplankton communities - which are widespread in freshwater ecosystems - to impact or control the fecal indicator in water remains poorly understood.

In this study, we investigate the role of metazooplankton on the survival of *E. coli*. We first perform laboratory feeding experiments to investigate how metazooplankton, especially the filter-feeding Cladoceran *Daphnia*, could impact the fate of *E. coli* under different experimental conditions in synthetic and lake water microcosms. Then, we compare the grazing pressure of different metazooplankton species on *E. coli* in surface water microcosms. Finally, we describe the seasonal evolution of grazing on *E. coli* by natural metazooplankton and protozooplankton communities in lake water.

In synthetic water matrices, the model species *D. pulex* (32 ind.L^{-1}) ingested *E. coli* and increased its loss rates. Following a 1,000-fold reduction in *E. coli* initial concentrations, decay rates decrease from 1.65 d^{-1} to 0.62 d^{-1}, reflecting the lower probability of encounters between *Daphnia* and *E.-coli*. In lake water matrices with a *D. pulex* density ranging from 0 to 65 ind.L^{-1}, we observed that *E. coli* loss rates increased with *Daphnia* densities, reaching 0.47 d^{-1} in the presence of *D. pulex* at 65 ind.L^{-1}. Also, a significant portion of the *E. coli* population loss was associated with matrix-related factors - most likely due to predation by other bacterivorous biota and/or bacterial competition.

When simulating a fecal pollution event in water containing representative metazooplankton population densities, *D. magna* (36 ind. L^{-1}) showed a significant impact on the *E. coli* loss rate and reached 2.33 d^{-1}. At the same population density, *D. pulex* impacted the *E. coli* population with a loss rate between 0.99 and 0.62 d^{-1}. With *E. coli* loss rates of 0.21 d^{-1}, the small rotifer *Brachionus calyciflorus*, exerted a much lower predation pressure on the *E. coli* population even at densities of

500 ind.L^{-1}. Although additional investigations are warranted, the low impact of the rotifer most likely results from its smaller size and lower filtering rate.

Finally, we report a seasonal evolution of grazing pressures on *E. coli* in a freshwater bay. Variations in community grazing rates on *E. coli* appear to be complex to understand and may be linked to species composition and abundance but also to water temperature and the occurrence of cyanobacteria.

In conclusion, *Daphnia* appears to be an efficient filter-feeder for the removal of *E. coli* in water. However, *E. coli* loss rates depend on a variety of factors such as predator to prey ratio, size of the zooplankton (and their filtration rate) and the type of matrix and its components (biotic interactions). These results provide a more comprehensive view of the effect of zooplankton on *E. coli* bacteria within water bodies and help to explain differences that can be observed at various times of the year in natural aquatic environments. Data on grazing rates should prove helpful for the improvement of current fate and transport models.

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CHAPTER 1 INTRODUCTION

1.1 Fecal pollution of aquatic resources

Surface water contaminated by fecal contamination such as *Escherichia coli,* and *Enterococci* (ENT) is a public health concern and a prevailing global environmental issue. This contamination occurs because of droppings from wildlife, inadequate sewage treatment (human fecal pollution), agricultural runoff, or faulty septic systems (Harwood et al., 2000; Johnson et al., 2004). The fecal indicator bacteria (FIB), which include fecal coliforms, *Escherichia coli,* and *Enterococci,* are an indicator of fecal contamination of bodies of fresh water. Consequently, the presence of fecal contamination is an indicator that a potential health risks exist for individuals exposed to the contaminated water. Therefore, they are used as a standard of microbial contamination of recreational water quality and as well as drinking water (US Environmental Protection Agency, 1986). Water polluted by fecal pollution is still one of the most critical origins of both epidemic and endemic worldwide diseases in both developing and developed countries. Releasing wastewater into water bodies without appropriate treatment is one of the high-risk factors which leads to disease and infections from fecal contamination. Many bacteria, viruses, and protozoa are transmitted via fecal pollution resuling in waterborne diseases including gastroenteritis, hepatitis, diarrhea, and respiratory and skin diseases. Exposure comes from inhalation or ingestion, via either direct consumption (drinking) or recreational activities (WHO, 2004; Stirling, 2001).

Waterborne diseases are prevalent not only in developing countries, but are also a serious challenge in developed countries. In the United States between the period of 1986 to 2000, 5,905 outbreaks were reported, with 95 outbreaks associated with recreational water. (Pandey et al., 2014). An outbreak in Milwaukee, U.S.A. in 1993 was responsible for the infection of around 403,000 people, having led to an estimated 50 deaths. In Milwaukee, *Cryptosporidium* contaminated the water supply due to inadequate filtration of water from Lake Michigan. In Walkerton, Ontario, Canada in 2000, the *E. coli* O157:H7 and *Campylobacter* contaminated the municipal well water and this contamination resulted 2,300 people falling ill, and seven dead (Craun et al., 2010).

It is important to monitor for fecal pollution in water for the protection of public health. When analyzing water samples for microorganisms (pathogens) of public health concern, it is difficult, expensive and time-consuming to test directly for the presence of the large variety of disease

causing pathogens. Therefore, water is tested for indicator species (coliforms and *enterococci*), which may be present when pathogens are present, and occur in high enough concentrations that they will be above detection limits (Glassmeyer et al., 2005).

Total coliforms have long been used as bacterial indicators. Total coliforms are common bacteria in the environment. They are Gram-negative, oxidase-negative, non-spore forming rods that ferment lactose with gas production at 35–37 °C after 24 to 48 hours in a medium with bile salts and detergents (Cabral, 2010). Total coliform counts are not necessarily a measure of fecal pollution, because total coliforms include bacteria that are naturally present in the environment and are not of fecal origin. However, their presence in water environment gives an indication of the possibility of existence of disease-causing pathogens, such as bacteria, viruses, and parasites (WHO, 2008; Medema, 2003; Payment, 2003). In addition, fecal coliforms are coliforms that ferment lactose at 44.5 °C, in a medium with bile salts (WHO, 2008; Grabow, 1996; Medema, 2003; Payment, 2003). Fecal coliforms are bacteria that exist in the intestines and feces (fecal origin) and include bacteria such as *Escherichia coli* (*E. coli*) (Young, 1992).

One of the potentially pathogenic bacteria in contaminated water from untreated wastewater is *Escherichia coli,* and it is also a fecal indicator. *Escherichia coli* has long been considered the most suitable bacterial indicator of fecal contamination in drinking water, and the testing methods are well developed and standardized (Edberg, 2000; Snozzi, 2001). *E. coli* are gram-negative, non-spore forming, rod-shaped, facultatively anaerobic bacteria and can ferment lactose with gas production after 24-84h at 37°C (Bitton, 1980). Also, they are defined as thermophilic coliforms that produce indole from tryptophan (feature used to confirm the presence of *E. coli*), but are also defined as a coliform able to produce β-glucuronidase or GLUC (although taxonomically up to 10% of environmental *E. coli* may not). All over the world, *E. coli* is used as an indicator organism to identify water and food samples that contain unacceptable levels of fecal contamination (for example, no *E. coli* is acceptable in distributed drinking water). The presence of *E. coli* in samples has been used as an indicator of fecal contamination since the turn of the 20th century (Environmental Protection Agency, U. S. 2000) and its presence indicates that there is a heightened risk of the presence of other fecal pathogenic bacteria and viruses (ex. *Salmonella spp*. or Hepatitis A) (Brüssow, 2004; Atlas, 1993). Recreational water guidelines from the USEPA revealed that in terms of prediction of occurrence of gastrointestinal illness in freshwater, the *E. coli* bacteria was a more reliable and consistent indicator than *enterococci* in terms of predicting illnesses (Wade et

al., 2003; U.S. EPA 2002). Compared to other coliforms, *E. coli* is shed in larger quantities, which facilitates its detection in water, soils, or food. In addition, *E. coli* O157:H7 and other strains of toxigenic *E. coli* are responsible for gastroenteritis that can cause effects and symptoms such as diarrhea, abdominal cramps, severe bloody diarrhea, and vomiting (Arnone and Walling 2007; WHO, 2003).

As the general test for coliforms includes bacteria that are not from fecal origins (e.g. *Enterobacter*, *Klebsiella*, *Citrobacter*), *E. coli* is considered a more specific indicator of fecal contamination. Therefore, to distinguish the *E. coli* from non-fecal coliforms, testing of the lack of an enzyme (selective for the *E. coli* organism) is required to confirm presumptive fecal coliforms or by using elevated temperature (44.5°C) (Francy et al., 1993; EPA). Furthermore, several studies (Abreu-Acosta and Vera, 2011; Wu, 2011 and Molleda 2008) have shown that the bacterial indicator organisms, including *Escherichia coli*, total coliforms, and fecal coliforms have limitations in their use to regulate and monitor pathogen levels in treated wastewater and the environment because of their lack of correlation with pathogens. As an example, Marino et al., 2005 studied the influence of the temperature and pH on *E. coli* concentrations. *E. coli* decreased with the increasing of pH and temperature, while *Vibrio cholerae* and *Enterococcus faecalis* increased. This difference showed why *E. coli* are sometimes not well correlated with *V. cholerae*. In drinking water distribution systems, the existence of a single positive sample for total coliforms is not enough to trigger public health action. Rather, if more than 5% of samples are positive, then actions and notifications are required (Edberg, 2000).

1.2 Regulations of drinking and recreational water quality

The bacteria in the coliform group (total coliforms, thermotolerant (faecal) coliforms, *E. coli*) and the *enterococci* have been used to monitor drinking and recreational waters for the presence of faecal contamination. However, testing for every possible waterborne disease causing microorganism would be prohibitive in terms of the financial resources necessary, the time required to perform the analyses, and the difficulties of isolating and quantifying. In addition, testing requires a proper laboratory, specialized equipment, and highly trained and experienced microbiologists. Therefore, authorities monitor for non-pathogenic faecal indicator bacteria that are present in high numbers in both human and animal faeces, which are indicative of faecal contamination and suggest the possible presence of enteric pathogens (Health Canada, Guidelines

for Canadian Recreational Water Quality – Third Edition). Outbreaks of illness associated with recreational water use result from exposure to infectious pathogens in recreational water venues that can be treated (pools and hot tubs or spas) or untreated (lakes and oceans). The outbreaks of illness associated with untreated recreational water can be caused by *Cryptosporidium* or by *Escherichia coli* (E. coli O157:H7 or E. coli O111) (Hlavsa et al., 2015). Also, drinking water contamination events can cause disruptions in water service, resulting in a large impact on public health. Waterborne disease outbreaks can be caused by Legionella or the parasite *Cryptosporidium* (Benedict, 2017).

In Canada, for recreational waters, the indicator organism used for primary contact activities is *Escherichia coli* (*E. coli*) for fresh waters and *Enterococci* for marine waters (Guidelines for Canadian Recreational Water Quality – Third Edition). For drinking water, *E. coli* is currently the best available indicator of recent faecal contamination in drinking water systems. *E. coli* can be used to provide an indication of the magnitude of a problem and thus inform the public health response (Health Canada, Guidelines for Canadian Drinking Water Quality). Also, in the province of Québec Canada the *E. coli is* the preferred bacterial indicator. The regulations for drinking water and recreational water (treatment and distribution in Québec) are based upon *E. coli* concentrations. (Ministère du Développement durable, de l'Environnement et de la Lutte contre les changements climatiques, Québec (MDDELCC, 2017); Regulation respecting the quality of drinking water (Q-2, r.40)).

1.3 Zooplankton (*Daphnia spp*) role in food web in water bodies

Daphnia spp has been well recognized as a keystone species in food webs and its important role as a primary consumer in aquatic food chains. The Cladocerans such as *Daphnia* species and protozoans are considered the major consumer of bacteria in freshwater lakes (JiJRGENS 1994; Pace 1990). *Daphnia spp* have shown to have the potential for reducing the microbial communities through several surveillance studies conducted. Therefore, this may lead to controlling the microbial population in natural waters (Siciliano et al., 2015).

The crustacean *Daphnia* has become a cornerstone of systems biology research for alternative testing and methodologies (Kurz and Ewbank, 2007). Also, in terms of measuring and screening toxicity, the use of *Daphnia* (*D. magna* and *D. pulex*) is considered a high sensitivity analytical

tool for screening toxicity of common environmental chemicals and monitoring of effluents and contaminated waters (Zhou et al., 2008 and Persoone et al., 2009). In addition, the ease of cultivating the crustaceans *Daphnia*, its well-studied biology, and its wonderful attributes of a short development time and large brood size make it suitable model for various environmental studies (Lambolez et al.,1994 and Seco et al., 2003).

The harmful effects of chemical treatment of wastewater makes ecological alternatives attractive; alternatives such as plants, invertebrate zooplankton, and fish that are able to remove algae and bacteria, and improve water quality (Shiny et al., 2005). In wastewater treatment, *Daphnia magna* filtration removed particles (diameters below 30 μm) efficiently removing *E. coli* bacteria. However, the removal depends on abiotic parameters such as water temperature and the hydraulic retention time (HRT) (Serra et al., 2014). Also, the filtration of *Daphnia magna* efficiently removed suspended sludge particles and was efficient at wastewater disinfection (Serra et al., 2016). *Daphnia magna* improved the water quality of wastewater, reducing the particle volume concentration of small particles. Experiments were performed in both the laboratory and in a mesocosm system (Pau et al., 2013).

From all material mentioned previously, we can illustrate the importance of zooplankton and their possible ability to impact the pathogenic bacteria such as *E. coli* in natural waters.

1.4 Thesis organization

This thesis is separated in the following manner:

Chapter 1 introduces the concepts of water fecal pollution and regulations of drinking and recreational water quality in Canada, as well as some basic information about the role of Daphnia spp in water bodies.

Chapter 2 provides the literature review concerning the fate and transport of E. coli in water, and biotic interactions between E. coli and zooplankton communities.

Chapter 3 contains the results.

Chapter 4 presents the experimental techniques and methods used in this work.

Chapter 5 presents the discussion.

Chapter 6 presents the conclusions and research perspectives.

CHAPTER 2 LITERATURE REVIEW

A large body of knowledge exists on the grazing of protozooplankton on planktonic or non-pathogenic microbial communities in water (Sheer and Sheer, 2002). Also, it is well known that protozooplankton species are reservoirs and vectors of pathogenic microorganisms in the environment (Barker and Brown, 1994; Greub and Raoult, 2004). In contrast, similar knowledge on metazooplankton is rather limited (Bichai et al., 2008; Nowosad et al., 2007). Rotifers and cladocerans have a cosmopolitan geographical distribution, are among the most common biota of freshwater ecosystems, and they are both capable of significant clearance of their planktonic preys in natural environments. Among the rare studies on metazooplankton-pathogen interactions, rotifers have been shown to ingest *Cryptosporidium* oocysts and *Gairdia* cysts (Fayer et al., 2000; Trout et al., 2002; Stott et al., 2003). Connelly et al. (2007) provide evidence that the cladoceran *D. publicaria* has the potential to substantially decrease the number of infectious *Cryptosporidium* and *Giardia* in freshwater. In laboratory conditions, Schallenberg et al., 2005 also shows that *D. publicaria* can remove *Campylobacter* from water. Ingestion of microorganisms is also suspected to modify their settling rates upon enclosure into fecal pellets (Brookes et al., 2004). However, the number of field studies reporting zooplankton predation on fecal pathogens and indicators is limited (Nowosad et al., 2007; Bichai et al., 2010).

2.1 Fate and transport of *E. coli* in water

E. coli is the most widespread indicator of fecal pollution, and it is very important to understand its survival and transport in water. In order to assess the severity of contamination for making appropriate management decisions, it is essential to understand *E. coli* survival rates in the aquatic environment (Blaustein et al., 2013). Additionally, to assess the risk of pathogens, it is recommended to understand critical variables influencing their fate and distribution in water (lakes and reservoirs). Sampling for *E coli* should be paired with other biological, physical, and chemical parameters, because several factors control the transport, distribution, and inactivation of pathogens in natural water environments (Brookes et al., 2004).

Several biotic and abiotic environmental factors influence the survival (fate) of allochthonous bacteria. Biotic factors such as grazing by protozoa is one of the main biological processes able to control allochthonous bacteria and their density (Van Limbergen et al.,1998). The control of

bacteria then depends on the digestion capacity of the grazer, and the bacterial concentration (Barcina et al.,1997). With regards to abiotic factors, physicochemical stress can lead bacteria to enter to a viable but non-culturable state or dormancy. Also, temperature, nutrient scarcity, visible light, and osmotic stress all have negative influences on the survival of bacteria. For instance, in aquatic systems the UV light has a negative effect by increasing the loss of culturability and the formation of active but non-culturable cells (e.g. Barcina et al.,1997). Menon et al. (2003) reported that in the North Sea, mortality rates of both autochthonous and fecal bacteria increased with the increase of temperature. Part of this mortality may be due to the grazing of a larger community of protozoa (heterotrophic nanoflagellates), which also rose with temperature increases. Wcislo and Chrost (2000) reported that in the aquatic environment, the disappearance of *E. coli* was related to the grazing of microflagellates. Also, the grazing of flagellates on bacteria was "size-selective" and *E. coli* with sizes of (0.5-2 μm) were more susceptible to predation than other small autochthonic bacteria. They also reported the effect of bacteriophages on the survival time of *E. coli* and found it to be insignificant. Their results also confirmed the influence of other factors on the survival of *E. coli* in aquatic ecosystems including other heterotrophic bacteria and the impact of temperature.

Sampling and measuring *E. coli* must be representative of environmental conditions. Therefore, it is necessary to understand detailed processes governing *E. coli* survival, including predation, which has been identified as a dominant process.

2.1.1 Temperature

Activity and abundance of the free-living bacteria is strongly influenced by temperature, one of the most important environmental factors affecting the transport and survival of bacteria (Peierls and Paerl, 2010). Bacteria in fresh waters survive longer at low temperatures than at high temperatures (Barcina et al.,1986). Despite other environmental factors controlling *E. coli* survival rates, such as sunlight intensity, pH, predation, and salinity, temperature is considered as the major factor influencing *E. coli* survival in water (Blaustein et al., 2013). At various temperatures in filtered and unfiltered river water microcosms, the mortality of *E. coli* increased with temperature (Flint, 1987). Likewise, *E. coli* and *Enterococcus spp* had significantly higher inactivation rates at 20°C than at 14°C (Noble, 2004).

On the other hand, The *E. coli* population structure in Dunes Creek was more varied in summer than in winter although the reasons remained unclear (Whitman et al., 2008). Temperature also

affects the survival of bacteria through its influence on other environmental factors, and by controlling the metabolic rate of bacterial cells (Jones, 1971; EPA, 1985). For instance, the influence of temperature on *E. coli* survival can be enhanced through the predatory activity of protozoa (Barcina et al., 1986a, b), but is also directly linked to temperature in the absence of eukaryotes (Anderson et al, 1983). Temperature and light penetration in natural environments are related. For example, a temperature gradient often occurs in natural aquatic environments. In the absence of light (for example, in darker, turbid waters) enteric bacterial survival is enhanced (Gould and Munro, 1980; Evison and Morgan, 1982), and the mortality of coliform bacteria is more likely to increase as temperature increases (Gameson and Gould,1985). Blaustein et al. (2013) reported that the *E. coli* in different water sources had highly variable survival rates at the same given temperature. This is potentially due to variations in biological and physical survival factors. Also, they demonstrated that the inactivation of *E. coli* in rivers and wastewater was faster than in lakes and other cleaner water sources.

2.1.2 Advection and sedimentation

Horizontal and vertical processes in water are important in determining the distribution of particles, fecal indicator bacteria, and pathogenic microorganisms. Horizontal transport in lakes and reservoirs is driven by wind and internal waves, which also generate vertical movements (Justin et al., 2004). In rivers, horizontal transport is primarily through advection. With precipitation, pathogens can be transported into rivers, lakes, and reservoirs, from runoff and inflow. In lakes, inflows may sink to lower depths, depending on temperature and salinity gradients, and flow along the bathymetry towards the deepest point. If pathogens are present in the inflow, they are diluted, and if they reach the deepest points where it is cold and dark, some pathogens such as *Cryptosporidium* oocysts can survive for extended periods (Justin et al., 2004; Deen et al., 2000).

Vertical transport of fecal indicator bacteria or pathogens occurs through settling processes. The aggregation of pathogens with particles containing organic matter plays an important role in the distribution and transportation of pathogens and survival in water environments. (Belle and Gerba, 1979). Aggregation may allow a consortia of bacteria cells to be more resistant to the effects of surrounding environmental stressors, then to transport and colonize in the host (Gilbert et al., 1993). Surface charges play a key role in pathogen–particle interactions in aqueous system (Ongerth and Pecoraro, 1996). For instance, in surface waters, *Cryptosporidium* oocysts may have negative

surface charges, the same as gatherings of natural particles, and in the same way as responding metal salt coagulation (Ongerth et al., 1996). Moreover, the sedimentation occurs when microorganisms attach to particles and settle in the sediments. Indicator organisms can persist in sediments, and may use the sediment to provide protection from environmental stressors (Anderson, 1983; Fish, 1995). In the mesocosms experiment by (Fish, 1995), the density of *E. coli* increased over the sample time when the mesocosms contained sterile water and sediment, probably due to the lack of environmental stressors. While, *E. coli* concentration decreased when the mesocosms contained only sterile water.

The size and density of particles are important factors in estimating the settling rate and the vertical distribution of pathogens. Particulate material may assist with the aggregation of pathogens, thereby influencing the rate of pathogen settling, but could also influence the predation of pathogens (Reynolds, 1984). It is important to consider particle size ranges when determining the settling and resuspension characteristics of pathogens (Brookes et al., 2004). The settling speed of a single oocyst in water was about 0.03 m day^{-1}, and it increased when it was associated with biologically treated sewage effluent particles (Medema et al., 1998). In addition, in their sedimentation kinetics, *G. lamblia cysts* and *C. parvum oocysts* could be qualified by oocysts size and density, as well as the density and viscosity of the sedimentation medium. The movements of oocysts in water occur more easily because of water flow, wind, temperature, and movement of aquatic organisms than because of gravitational settling (Medema et al., 1998). In the Lake of Burragorang in Sydney, Australia, field measurements show that the estimation of sedimentation rates of oocysts is around 5–10 m/day-1, meanwhile, the free-floating oocysts settle very slowly. Consequently, oocysts need to be attached to particles to have high settling velocities, which are necessary for modelling pathogen transport (Hawkins et al.,2000).

2.2 Biotic interactions between *water bacterial communities* and zooplankton communities

Although much has been described about the abiotic stressors and factors are controlling the fate of pathogens and indicator organisms (viability and/or infectivity) in water, such as temperature, solar radiation (UV and visible), nutrient scarcity, salinity etc., quite limited information exists on the biotic effects (Blaustein et al., 2013; Wanjugi and Harwood, 2013).

2.2.1 The zooplankton including the filter-feeder *Daphnia*

What are zooplankton? Zooplankton are small animals that float freely in the water column of lakes and oceans, depending on water movement. The sizes of zooplankton community of most lakes ranges from a few tens of microns (Protozoa) to up to 2mm (macrozooplankton). The dominant groups of zooplankton in most lakes are protozooplankton, which is the protozoa, and metazooplankton, which are rotifers and crustaceans (*Cladocerans Daphnia* and *Copepods*). Zooplankton play a important role in water-based food webs, they can be food for fish or predators, grazing on algae, bacteria, protozoa, and other invertebrates (Vanni,1988).

One important zooplankton is the *Daphnia*. *Daphnia* - better known as water fleas - are characterized by flattened leaf-like legs that are used to produce a water current to act as a filtering apparatus, and are members of the order *Cladocera*. They have an enclosed body in an uncalcified shell or carapace (1-5 mm long), and are ubiquitous in freshwater aquatic environments (Ebert, 2005). In addition, *Daphnia* can feed on algal blooms (for proteins and carbohydrates), bacteria, and yeast. The *Daphnia* life cycle starts with an egg, then progresses to juvenility, adolescence, and adult life, with an average lifespan of between 40-56 days (Pennak, 1989). In normal environmental conditions, *Daphnia* have the ability to rapidly clone themselves asexually. However, when environmental conditions deteriorate, such as a lack of food availability or poor temperatures, *Daphnia* can procreate sexually and produce resting cysts (eggs), which are able to hatch when conditions improve (Figure 2.1).

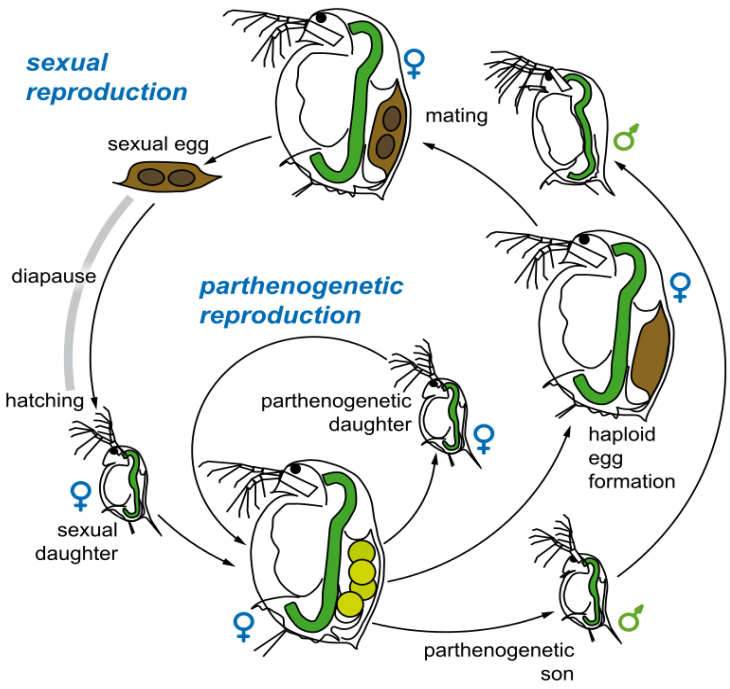

Figure 2. 1 : The life cycle of the cladoceran *Daphnia pulex* (asexual and sexual phases).

Daphnia are important because they have an important role as a model for ecological studies and are an essential part of the food web in water bodies. They have been well recognized as a keystone species in food webs, and for their important role as a primary consumer in aquatic food chains. The Cladocerans such as the *Daphnia* species and protozoans are considered to be the major consumer of bacteria in freshwater lakes (Jijrgens 1994; Pace 1990). Also, evidence based on grazing phenomenon suggests it's one of the major forces shaping the bacterial community structure in waters (Güde, 1989). In addition, *Daphnia* are often used in the environmental monitoring of the aquatic environment and in bioassays. For instance, the Environmental Protection Agency (EPA) recommended *Daphnia magna* and *Daphnia pulex* as standard aquatic test species for toxicity tests. Moreover, compared to other zooplankton, the *Daphnia* organisms are more intensive grazers (filter feeders) and they are widely available in many surface waters like ponds. *Daphnia* have the potential as a community to filter a large volume of water (Cyr et al., 1992). Also, *Daphnia* can be cloned naturally and thus, the culturing of *Daphnia* is easy and cost-efficient (Weber, 1991 and EPA, 2002). In general, *Daphnia* are among the best-studied subjects in ecology literature (Ebert, 2005).

2.2.2 Some interactions between zooplankton (*Daphnia*) and bacterial communities including *E. coli*

Generally, the bacteria that have been ingested by zooplankton might be either digested (although yet unproven) or excreted out back into the water environment. Regarding the predation by indigenous biota and their effect on *E. coli* survival in water, a lot of information has been gathered for protozooplankton (heterotrophic nanoflagellates (HNF) and ciliates). However, much less is known for the role of metazooplankton grazing (Bichai et al., 2008).

Regarding the association of bacteria with zooplankton in aquatic systems, bacteria in general may attach to zooplankton either directly to the exoskeleton (Nagasawa and Nemoto, 1988) or to other particles such as food particles or aggregates (Simon et al. 2002, Carrias and Sime-Ngando 2009). In addition, bacteria may be found inside the gut of zooplankton due to ingestion (Tang, 2005). While there is a lack of information on zooplankton being physically associated with pathogenic microorganisms in water, bacterial settlement of the exoskeleton surface of zooplankton organisms and attachment to planktonic animals is more validated as a mode for pathogens to be transferred from the water phase in natural systems. Certain bacteria can attach themselves to the surface of zooplankton organisms where they discover a microhabitat that enables them to persist longer in the environment. So far, the most widely known example is the case of *Vibrio cholerae*, the bacterium responsible for cholera (Cottingham et al. (2003). Tang et al. (2010) reported that bacteria interact with zooplankton in several different ways, with implications for microbial production, evolution, biogeochemical fluxes, diversity maintenance, and dispersal.

Knowledge of the grazing effects of metazooplankton on *E. coli* is still limited as compared to protozooplankton, which is one of the main biological processes controlling allochthonous bacteria and their concentrations in water (Barcina et al.,1997). Menon et al. (2003), reported that in river and in the coastal areas, protozooplankton grazing was responsible for more than 90% of the overall mortality rate of fecal and autochthonous bacteria. In a constructed reservoir, microflagellate grazing was found to be the main factor responsible for *E. coli* mortality, and the microflagellates were more size-selective of *E. coli* cells (Wcislo and Chrost, 2000). In addition, in lake ecosystems, protozoans or metazoans have been shown to play a key role as bacterial consumers depending on the structure of the food web. Heterotrophic nanoflagellates (HNF) can change the bacterial community such that smaller cells dominate because they selectively consume the large-sized

bacterial cells (Jürgens at al., 1994). Grazing information is still limited to a few zooplankton groups and environments, for example, copepods in coastal and estuarine waters (Menon et al., 2003). There are few studies that have examined microorganism survival during ingestion by zooplankton species. These studies generally do not provide any information on water-borne pathogens that zooplankton grazing in natural conditions with realistic concentrations (Menon et al., 2003; Wcislo and Chrost., 2000; Barcina et al., 1997 and Jürgens et al.,1994).

However, a few studies show the effect of metazooplankton on *E. coli*. A feeding rate experiment of *Daphnia magna* was done by McMahon and Rigler (1965). *Daphnia magna* in different concentrations (50, 100 and 20 ind/mL) fed on four different foods (*E. coli*, *Saccharomyces cerevisiae*, *Chlorella vulgaris*, and *Tetrahymena pyriformis*). The feeding behavior *of Daphnia magna* was approximately similar for all conditions. Although maximum rates expressed as cells/hr-1 varied tremendously, the maximum volume of the different foods that were consumed were similar. The filtering rates for *E. coli* per *Daphnia magna* (2.8-3.3-mm size) was 5.6 million cells/hr. However, filtering efficiency was independent of the size of food (ranging from 0.9 μ^3 to 1.8 x 10^4 μ^3).

McMahon and Rigler (1963) studied the crustacean *Daphnia magna* incubated with two foods (*Chlorella vulgaris* and *Saccharomyces cerevisiae*). The feeding rate was proportional to the concentration of food, although there were critical thresholds above and below which little effect was observed on feeding rates. There exists an effect on the feeding rate of *Daphnia* by the food present in their gut before starting an experiment. *Daphnia* pre-fed at a very low rate do, for a short time, feed more rapidly than those prefed at a higher rate. However, this study did not observe a decrease in feeding rate with time (McMahon and Rigler, 1963). However, Hadas et al. (1983) reported that *Daphnia magna* is able to ingest bacteria and algae, also a cell-free extract (CFE) of *Daphnia magna* was able to lyse *E. coli* cells by causing damage to *E. coli* cells as represented in the release of the enzymes of the bacterial cell.

Cladocerans, especially the *Daphnia* genus, are important in terms of filtration capacity. They are able to ingest pelagic food particles (including bacteria) over a wide range of sizes by collecting them with their thoracic appendages (Brendelberger 1991; Riemann 1985). Also, Cladocerans like *Daphnia* are widespread and found in many lakes and ponds over the world. Their presence can

change the structure and performance of microbial food webs in freshwater ecosystems. *Daphnia* can apply significant grazing pressure on all components of microbial food webs (picoplankton, nanoplankton and microplankton) because they are able to filter a large spectrum of particle sizes (Jürgens 1994). In terms of ingestible particle sizes by *Daphnia*, the planktonic bacteria are at the lower level, while Nanoplanktonic protozoans (flagellates and small ciliates) are in the optimum size range. Thus, *Daphnia* could become the main bacterivores and consume the major portion of bacterial production. Also, *Daphnia* grazing can have a positive influence effect on bacterioplankton (Jürgens 1994). In terms of Cladocerans filler mesh size and its capability of retention of very small particles, four Cladocera species (*Daphnia magna*, *Daphnia pulex*, *Simocephalus vetulus*, and *Diaphanosoma brachyurum*) had retention efficiencies of between 33 and 154% of food particles (consisting of green algae, free-living planktonic bacteria and cyanobacteria). Although, the Cladoceran species with coarser filtering meshes showed lower retention efficiencies for bacteria and cyanobacteria, the Cladocerans can utilize free-living, single-celled, planktonic bacteria as a resource of food (Brendelberger. 1991). In addition, Degans et al., (2002) reported that there was a quick change in structure and biomass bacterioplankton community upon exposure to *Daphnia* grazing and heterotrophic nanoflagellates (HNF). He concluded that *Daphnia* have a clear impact on the lake bacterioplankton, either by direct grazing on bacteria or grazing on protozoan bacterivores. *Daphnia magna* lived on diets containing small proportions of heterotrophic bacteria (*E. coli* 20% and Flavobacterium ≤ 50%) and *Daphnia* grew significantly in these diets compared to pure algal diets, suggesting the occurrence of nutritional upgrading by these bacteria. Thus, the nutrition of *Daphnia* may have improved with the presence of the more diverse bacterial composition (Freese and Martin-Creuzburg., 2013). Also, high biomass of *Daphnia pulex* can sufficiently keep the bacterioplankton below the carrying capacity, since the bacteria growth was about one doubling per day and the *Daphnia* was able to balance it by the grazing activity (Jürgens at al., 1994).

In freshwater habitats, there is a complex interaction between protozooplankton communities and metazooplankton communities because *Daphnia* can feed upon protozooplankton (ciliates and flagellates) (Jürgens at al., 1994). In the study of Jürgens at al. (1994), they reported that the *Daphnia pulex* predation and grazing on the protozoans (nanoflagellates) was much higher than on bacteria. When the *Daphnia pulex* were removed, the heterotrophic nanoflagellates developed and

became the main bacterivores. This means that when large *Daphnia* populations are present, the heterotrophic nanoflagellates become unimportant.

Daphnia spp, have the potential for reducing some bacterial communities, as demonstrated through surveillance studies. The presence of *Daphnia* can lead to the control of the abundance of microbial communities in natural waters (Siciliano et al., 2015). Under artificial experimental conditions, *Daphnia pulicaria* could naturally control *Giardia lamblia* cysts (zoonotic parasites) and *Cryptosporidium parvum* oocysts by controlling their density, viability, and infectivity (Connelly et al., 2007). In addition, *Daphnia carinata* and *Campylobacter jejuni* were incubated for 72 hours in simulated and natural aqueous conditions. The predation of *Daphnia carinata* caused death and reduced the *Campylobacter jejuni* concentrations, as compared to the control without *Daphnia carinata*. The reduction in the *Campylobacter jejuni* concentration was 2 logs (Schallenberg et al., 2005). Moreover, an experimental study on controlling pathogens in nature demonstrated that the Cladocerans *Daphnia pulex* and *Moina macrocopa* reduced *Vibrio cholerae* densities more efficiently than rotifers (Ramirez et al., 2012). On the other hand, some authors reported that the filter-feeders (Cladocerans) are not able to digest all kinds of bacteria. Unlike rod shaped bacteria, general coccoidal bacteria survived the digestion of *Daphnia ambigua*. Therefore, perhaps "viable gut-passage" is one of the mechanism of bacteria to survive cladoceran grazing (King et al., 1991). However, even though there is no direct evidence of bacterial ingestion, digestion, or death by zooplankton, grazing of zooplankton on bacteria is expected to be an important factor in the reduction of bacteria in natural environments (Pace 1988).

2.3 Hypotheses

- In view of the filtration capacity of metazooplankton communities and the experimental evidence that they ingest FIB and pathogens with the potential to reduce their numbers (Schallenberg et al., 2005; Connelly et al., 2007), it is expected that grazing by metazooplankton, especially *Daphnia*, significantly contributes to the removal and potential inactivation of fecal bacteria in surface waters (Boehm et al., 2005).
- It is expected that the grazing pressure varies over the year since zooplankton population dynamics follow seasonal trends.

2.4 Objectives

This research has been organised into three specific objectives that have been adressed in this master's thesis:

1. Using the model species *D. pulex*, determine the impact of *Daphnia* grazing on *E. coli* under various experimental conditions
   - Under different effect of matrix (presence of algal food, type of water).
   - Under different *Daphnia* densities.
2. Assess the grazing impact of various metazooplankton species (*Daphnia*, *Moina* and *Brachionus*) on *E. coli*.
3. Quantify the grazing impact of natural zooplankton communities on *E. coli* and determine how this impact evolves seasonally.

CHAPTER 3 MATERIAL AND METHODS

Three different experiments with different designs and conditions, and with different zooplankton species were performed. The first experiment was done with only *Daphnia pulex*, either in synthetic water medium (ADaM), or lake water, to study the interactions *Daphnia- E. coli* upon exposure of the FIB to the grazer. The second experiment was done by *Daphnia pulex, Daphnia magna,* and rotifers *Brachionus calyciflorus*. Finally, the third experiment was done by using natural metazooplankton and protozooplankton communities (Figure 3.1).

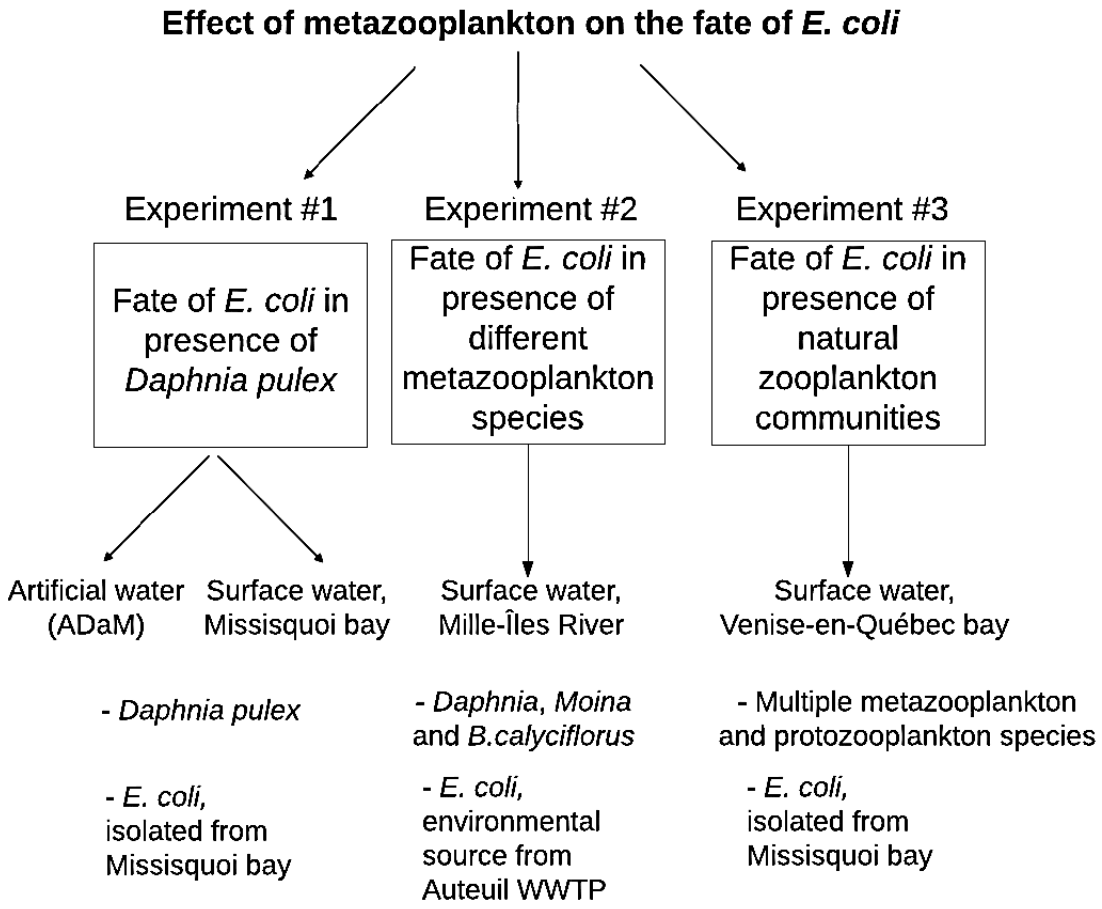

Figure 3. 1: experimental setup.

3.1 Maintenance of laboratory cultures

Before starting our experiments two cultures were maintained in our lab: Algae cultures and Zooplankton cultures. The algae cultures were used as food for the zooplankton cultures' growth, then the zooplankton cultures were used in our experiments to assess their impact on *E. coil* bacteria.

3.1.1 Algae cultures

Cultures from three algal species (*Scenedesmus quadricauda*, *Pseudokirchneriella subcapitata*, and *Ankistrodesmus falcatus*) were kindly provided by Prof. Melania Cristescu from the Department of Biology, McGill University and were maintained in modified BBM (Bold's Basal Medium, see appendix A seection 1.3) in 1L Erlenmeyer flasks with continuous stirring (magnetic stir bar) and air bubbling. The cultures were grown during a photoperiod of 18 hours of light and 6 hours of dark using triphosphorus fluorescent lights at an ambient temperature of 20 ± 1°C. The volume of culture medium was changed on a weekly basis. The harvest of algae cultures was done during the log phase after 7 to 8 days growth. Liquid algae cultures were distributed into 250mL centrifuge flasks and centrifuged at 4000rpm (3350*g*) during 10 minutes. The supernatant was discarded to remove the waste products generated by the algal metabolism during growth (Figure 3.2).

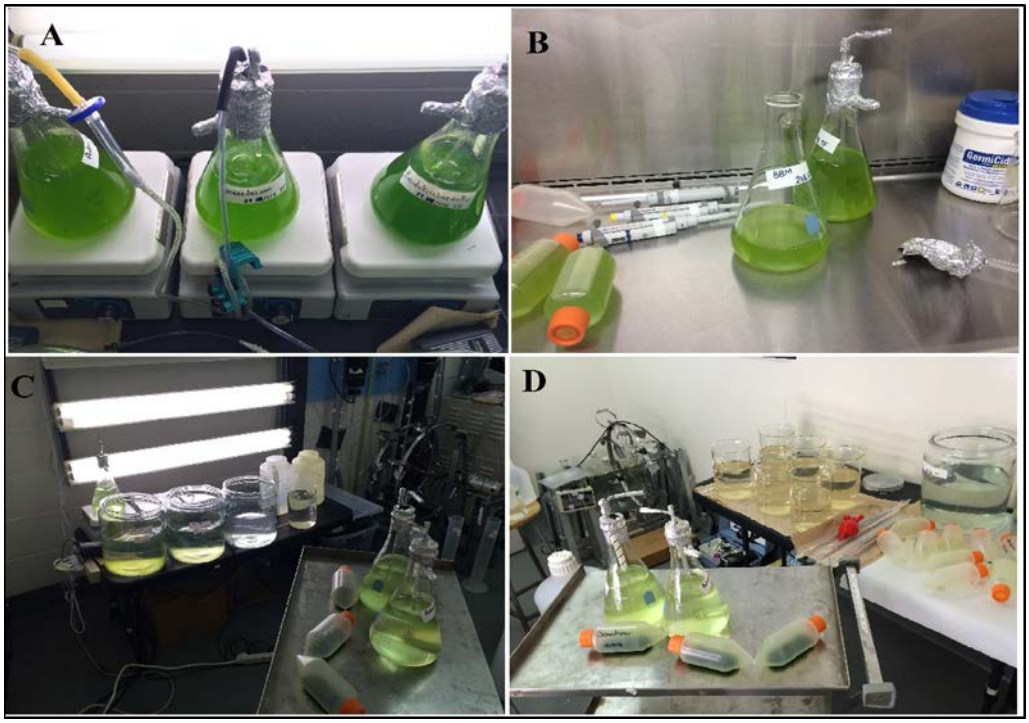

Figure 3. 2: A) algal cultures of *Scenedesmus quadricauda*, *Pseudokirchneriella subcapitata*, and *Ankistrodesmus falcatus*. B) harvesting of algae culture in 250 mL bottles under laminar flow cabinet before centrifugation. C and D) 1L Erlenmeyer flasks inoculated with freshly harvested algae for re-culturing.

The algal pellet was resuspended in BBM medium and the algae concentration was determined using a Neubauer counting chamber (Figure 3.3). A 1000-fold dilution of the resuspended pellets was prepared by successive Log_{10} dilutions. Then, approximately ~200 µL of the 1000x dilution was transferred to the counting chamber, covered with a coverslip and placed under an epifluorescence microscope (Olympus, 10x or 20x magnification) to count algal cells. The average of at least two counts was taken and calculated using the following formula to estimate the algae concentration.

$$C = (N / V) \times d \qquad (1)$$

where C is the stock concentration (expressed in cells. mL^{-1}), N is the average number of cells counted from min. two "16 squares-areas", V is the volume of one square and d is the dilution factor.

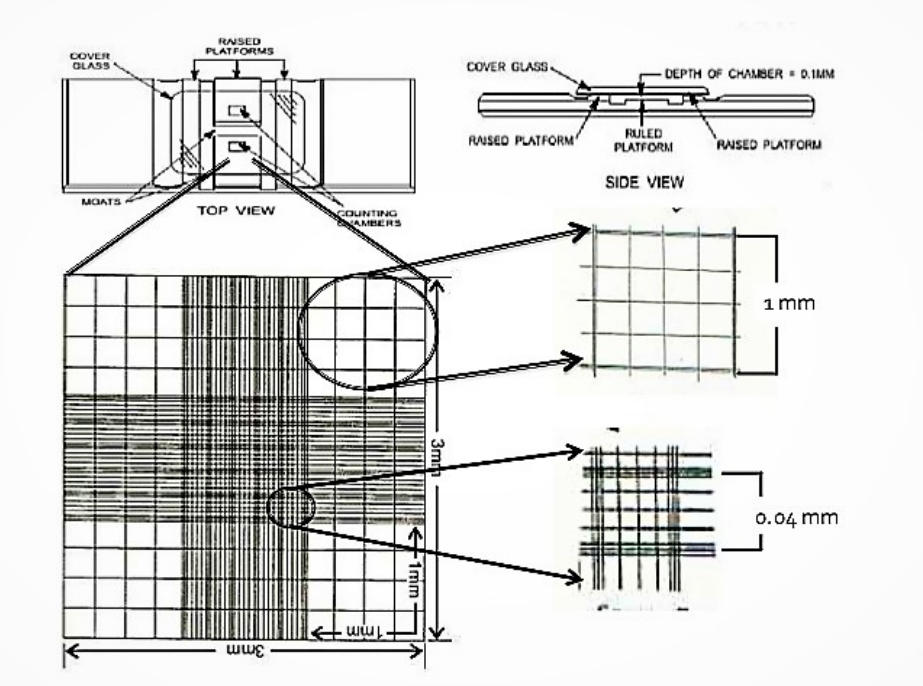

Figure 3. 3: Neubauer counting chamber, (www.slideshare.net).

Finally, 1 mL of each algae species was used to re-culture them by inoculating them in new BBM medium (500 to 1000 ml), before pooling the freshly harvested algae and storing the stock at 4°C for daily feeding of *Daphnia* cultures.

3.1.2 Zooplankton cultures

3.1.2.1 Pictures of the model species

In this work, the following species were used to assess their impact on the decay rates of *E. coli* (*Daphnia pulex*, *Daphnia magna*, *Brachionus calyciflorus* and *Moina*) (Figure 3.4).

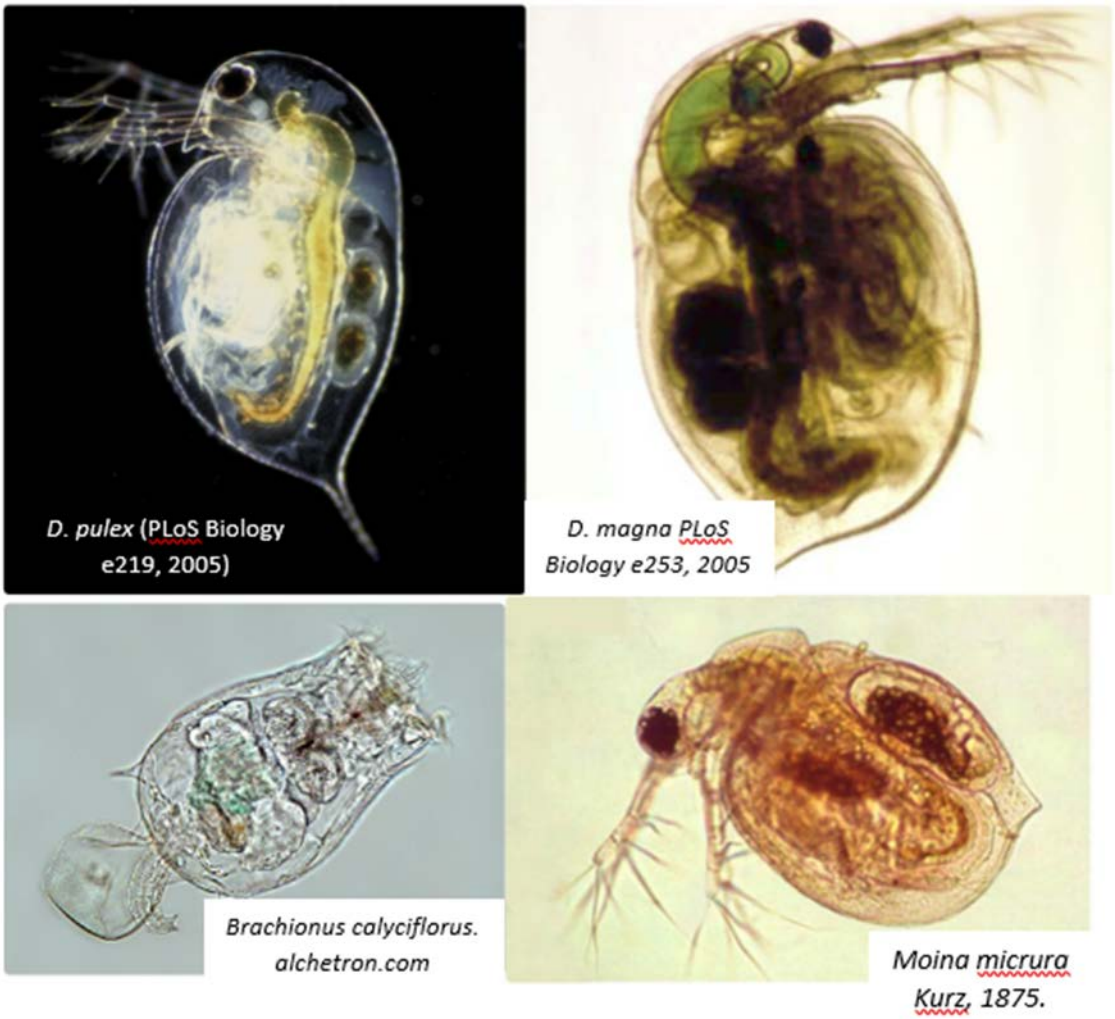

Figure 3. 4: Model species used during the present work

3.1.2.2 Daphnia

Daphnia pulex Leydig, 1860 were purchased from Carolina Biology Supply (Burlington, CA) and two species of *Daphnia magna* (clone1 "KLEINE" from Germany and clone 2 "XINB3" from Finland) were kindly provided by Prof. Melania Cristescu from the Department of Biology at McGill University. *Daphnia* was grown in Artificial Daphnia Medium (ADaM) (Kluttgen et al.,

1994), (see appendix A section 1.1). The cultures (approximately 6 to 7 L) (Figure 3.5) were maintained in the laboratory at 20°C and fed with the algae mixture (*Scenedesmus quadricauda, Pseudokirchneriella subcapitata, and Ankistrodesmus falcatus*). The daily amount of algae food varied from 1 to 2 ml per microcosm, which is about 10^8.mL^{-1} or 10^5.mL^{-1}. Dead *Daphnia* bodies, molts, and settled algae were removed regularly with small pipettes. The culture medium was changed every 7 to 10 days.

3.1.2.3 Brachionus calyciflorus

Resting eggs of *Brachionus calyciflorus* were purchased (www.brineshrimpdirect.com) for cultures of the model rotifer. Resting eggs were resuspended in tap water and the vial was shaken vigorously to hydrate the eggs, which were then poured into 20 mL freshwater (no chlorine) in a shallow wide petri dish that provided a high surface area to volume ratio allowing sufficient oxygen exchange. The dish was covered with a clear lid to reduce evaporation for 24 to 72 hours. After 24 hours, the eggs hatched and the rotifer juveniles were fed with a few drops of algae (*Scenedesmus quadricauda*) until the water produced a faint green color. At 48 hours, the culture was transferred into a 500mL beaker of EPA medium (see appendix A section 1.2) and fed with enough algae to produce a light green color. After 4 days, the culture was transferred to a 2L beaker of EPA medium and fed daily with *Scenedesmus quadricauda* following the instructions of the provider. Also, in the initial stage of rotifer culture, it was important to provide the food routinely but not in excess because dark green water could impede reproduction due to high pH. As for *Daphnia* cultures, rotifer cultures were maintained at 18 hours of light / 6 hours of dark periods at 20 ± 1°C.

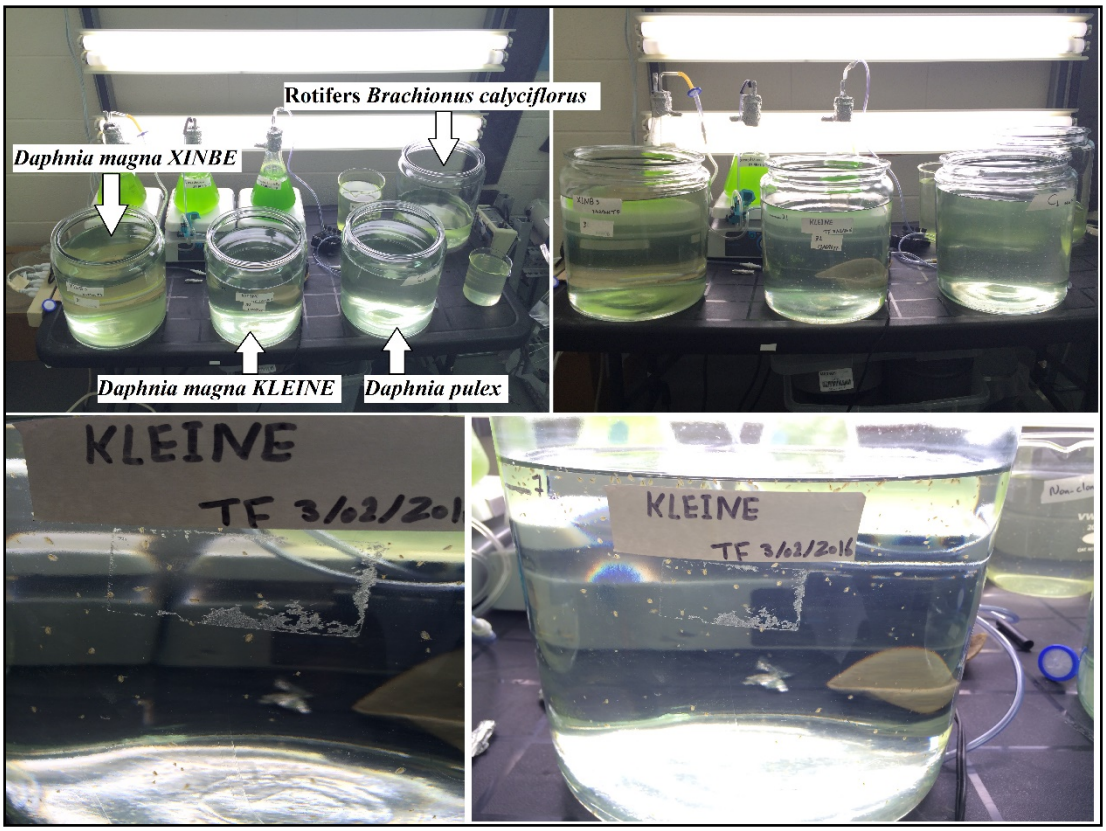

Figure 3. 5**:** Zooplankton cultures for *Daphnia pulex, Daphnia magna* (clones 1 or "KLEINE" and 2 or "XINB3") and *Brachionus calyciflorus***.**

3.1.2.4 Moina sp.

The crustacean *Moina sp.* was isolated from the Saint-Hyacinthe wastewater treatment plant (WWTP) from a secondary treatment basin (appendix A section 3). *Moina* individuals were collected with a zooplankton net (mesh size of 53 µm), and brought back to the laboratory where they were transferred to EPA medium and kept under the same conditions as *Daphnia* and *Brachionus* (20°C, 18:6 light-dark cycles). The diet for *Moina* consisted of the same algae mixture as for *Daphnia* supplemented with yeast (1 teaspoon of yeast dissolved in 500 mL of 100 degree tap water). The amount of yeast feeding was adjusted with the color of the culture and *Moina* was fed 3 to 4 times a week. (NFC: Moina - Intensive Culturing Method. GHemsath at alascom_att.com. Tue, 14 Sep 1999).

Unfortunately, due to the difficulties that were encountered in the maintenance of the *Moina sp.* culture the experiments were ended because of the high mortality of *Moina sp*.

3.2 Origin of *E. coli*

A water sample from Missisquoi Bay, a shallow transboundary bay of Lake Champlain (Québec, Canada) (Figure 3.6), was cultured on MI agar and single blue colonies were isolated, sub-cultured on Tryptic Soy Agar (TSA) during 24h at 37°C, and confirmed for indole production using Kovac's reagent. Confirmed colonies were then inoculated in Tryptic Soy Broth (TSB) during 24h at 37°C. Cells were washed by two centrifugal steps. The cell pellets then re-suspended in new TSB tube containing 0.22 µm-filtered glycerol (10-15% final v/v). Several 1mL aliquots of this suspension were then aseptically transferred to cryotubes and stored at -80°C before further use. A new aliquot of *E. coli* sister culture was used for every new experiment. To obtain a new *E. coli* concentration for new experiment, inoculating of new *E. coli* cells from the cryotubes on Tryptone Soy Agar (TSA) and incubated at 35°C during 18-20 hours. Then, some colonies were removed by the loop and re-suspended in sterile phosphate buffer. The suspension was adjusted to an OD_{600} of 1.0 (corresponding to ~10^9 $CFU.mL^{-1}$) by using a spectrophotometer (Unico SpectroQuest Model SQ2800 Single Beam UV/Visible Scanning Spectrophotometer).

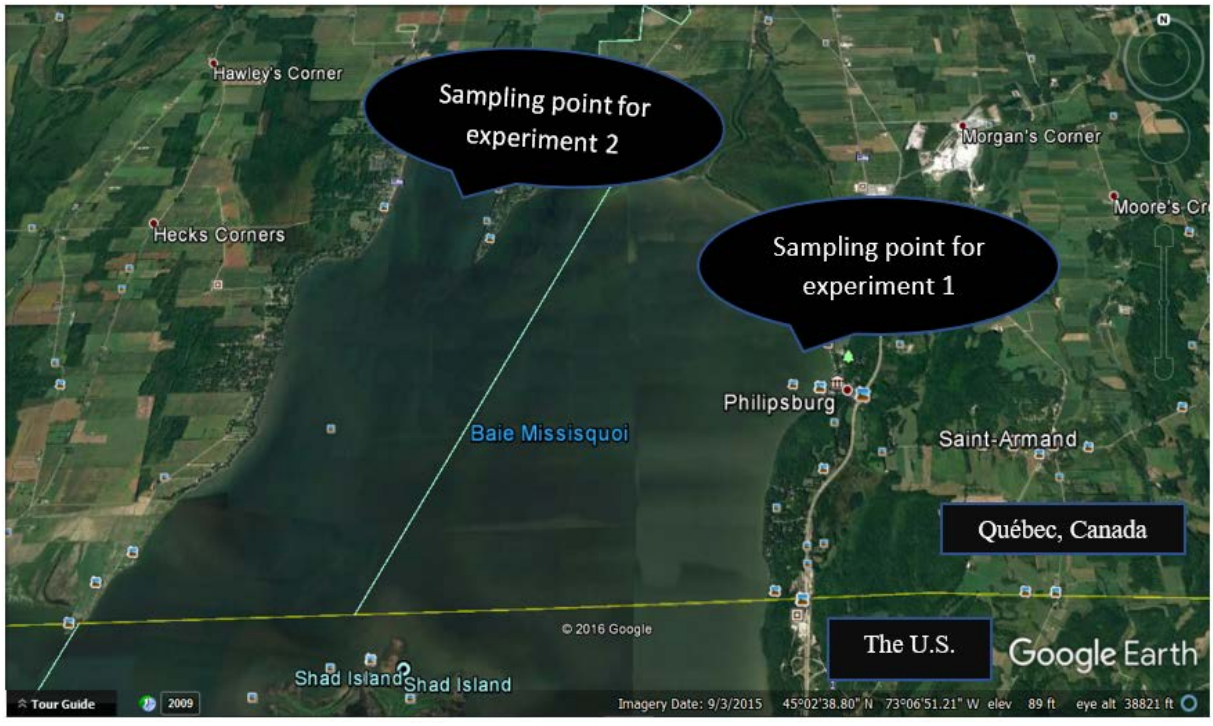

Figure 3. 6: The location of samples collection (Missisquoi Bay. Quebec, Canada). Sampling point for experiment 1 is "Philipsburg" and for experiment 3 is "Venise-en-Québec".

3.3 Impact of *D. pulex* on the decay rates of *E. coli*

3.3.1 Synthetic water

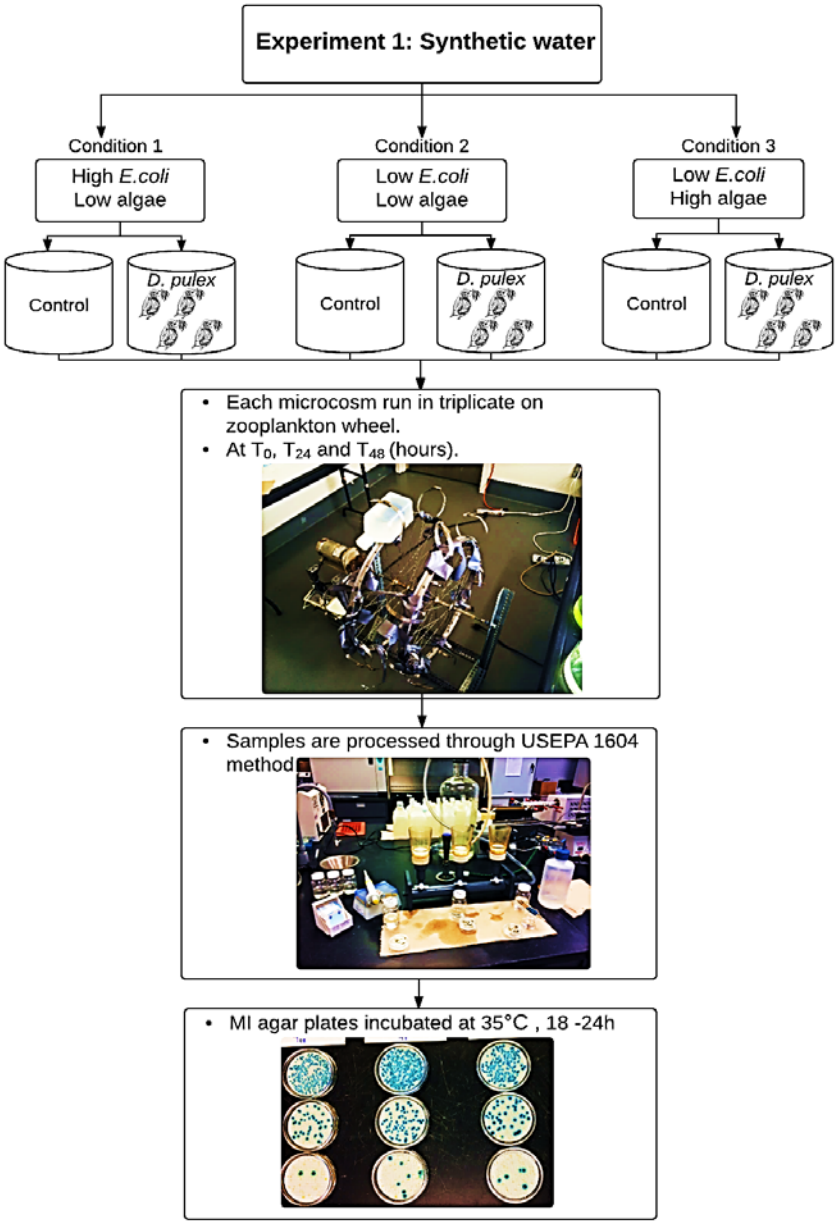

Figure 3. 7: Impact of *D. pulex* on the decay rates of *E. coli* in synthetic water (ADaM).

A new sister culture of the environmental *Escherichia coli* strain stored in TSB-glycerol at -80°C was cultured on Tryptone Soy Agar (TSA) and incubated at 35°C during 18-20 hours. Several colonies were harvested using a sterile inoculation loop and re-suspended in sterile phosphate buffer. The suspension was adjusted to an optical density (OD_{600}) of 1.0 (corresponding to ~10^9 $CFU.mL^{-1}$) using a spectrophotometer (Unico SpectroQuest Model SQ2800 Single Beam UV/Visible Scanning Spectrophotometer).

In order to determine *E. coli* decay rates in the presence or absence of *Daphnia pulex* under varying conditions (Figure 3.7), a total of 18 microcosms (3 conditions, carried out in triplicate, in the absence and in the presence of *Daphnia*) were filled with synthetic water medium (ADaM) for a total volume of 1.28L (bottles filled to the top). Control microcosms without *D. pulex* were performed to assess natural *E. coli* mortality. *Daphnia* microcosms contained 40 *D. pulex* juveniles of similar size (~1 mm). The green algae *Nannochloropsis atomus* was added to check how algae quantity would affect grazing of *D. pulex* on *E. coli*. *Daphnia* and control microcosms were run in triplicates and incubated during 48 hours on a zooplankton wheel (rotation at 1 rpm during 2 minutes every 2 hours) under controlled ambient conditions (20°C ±1) and a photoperiod of 18 h light / 6 h dark) (Figure 3.8).

Two different *E. coli* spike doses were tested to assess the effect of *E. coli* initial concentration on its decay rate. Two different algae concentrations were added to stimulate grazing (Schallenberg et al., 2005), also to test how algae amount would affect grazing of *D. pulex* on *E. coli*. The following table (Table 3.1) illustrates the details:

Table 3. 1: The concentration of *E. coli* and algae spike with the *Daphnia*.

Condition	*E. coli* (CFU/mL)	Algae (*Nannochloropsis atomus*). cells. mL^{-1}	*Daphnia* densities (ind./L)	
			Daphnia microcosm	Control microcosm
1	10^6 (High)	10^3 (Low)	32	0
2	10^3 (Low)	10^3 (Low)	32	0
3	10^3 (Low)	10^5 (High)	32	0

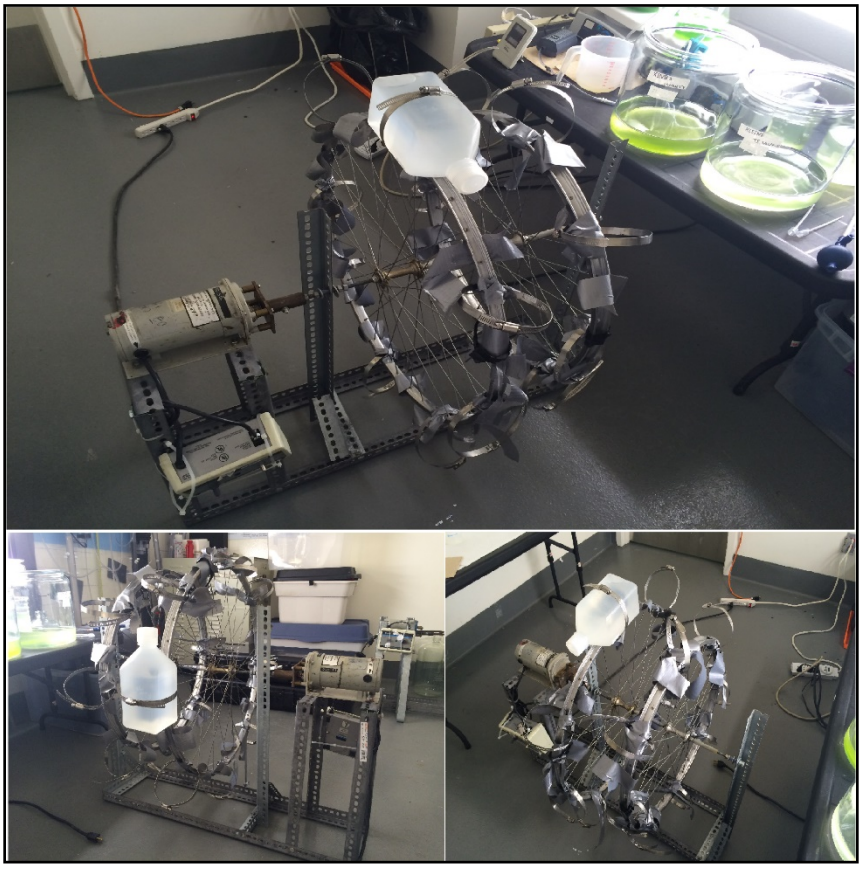

Figure 3. 8**:** Zooplankton wheel used for incubation of bottle microcosms during experiment 1.

Bottles were mixed manually by gentle up and down movements, giving special care to avoid any harm to *Daphnia.* A first sample (100 µL- samples or appropriate dilutions) was collected at (T_0) for the enumeration of initial *E. coli* levels, then the bottles were capped with parafilm and incubated on the zooplankton wheel. Following the same procedure, sampling was repeated after 24 and 48 hours (T_{24}, T_{48}). All samples were processed as described in section 3.8 (USEPA Method 1604, section 3.6) and for enumeration of culturable *E. coli*. By using *E. coli* counts (Figure 3.9), the loss rate (k) was calculated and derived from the following equation:

$$Ln\left(\frac{C_t}{C_0}\right) = -Kt \qquad (2)$$

where K is the decay rate, C_0 and C_t are the concentrations in culturable *E. coli* (CFU. mL $^{-1}$) at T_0 and T_{48}, respectively, and t is the time of incubation (days). The decay rate of *E. coli* followed first order kinetics between the incubation time of 0 and 48 hours (r^2 ranged between 0.77 and 0.96, $p<0.01$).

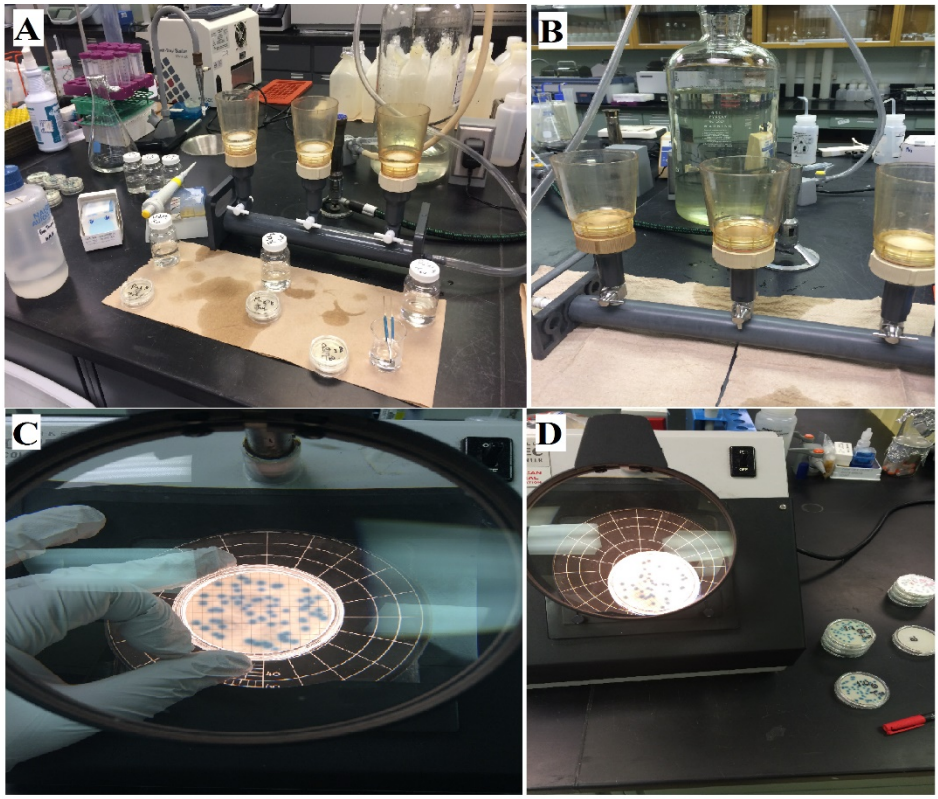

Figure 3. 9: USEPA method 1604 for enumeration of culturable *E. coli*. A and B) vacuum filtration ramp. C and D) Blue *E. coli* colonies on MI agar plates after incubation at 35°C during 18 - 24 h).

For both *Daphnia* and control microcosms that initially contained high algae concentrations (1.3×10^5 cells. mL^{-1}), algae were counted every 24 hours using a Neubauer counting chamber to estimate the filtration rate of *Daphnia*. A calculation of algae amount consumed by *Daphnia* during 24 hours was done after each count, then replaced by fresh algae from the stock suspension to keep a constant amount of food throughout the experiment. The calculated filtration rates were then used to estimate the theoretical removal rates of *E. coli* with the assumption that both *N. atomus* and *E. coli* were homogeneously distributed within the microcosm and filtered with comparable efficiency (McMahon and Rigler 1965). To estimate if there were any changes in cell concentrations due to non-grazing factors, algae were counted also in control microcosms.

During the first experiments of *D. pulex* (synthetic water), the *Daphnia* were checked and observed to determine if they were living or dead. *Daphnia* began to die off at 27 hours (see appendix section 2.1 , Figure A.1). Due to this mortality, experiments were ended after 48 hours instead of 72, which was determined to be too long.

3.3.2 Lake water

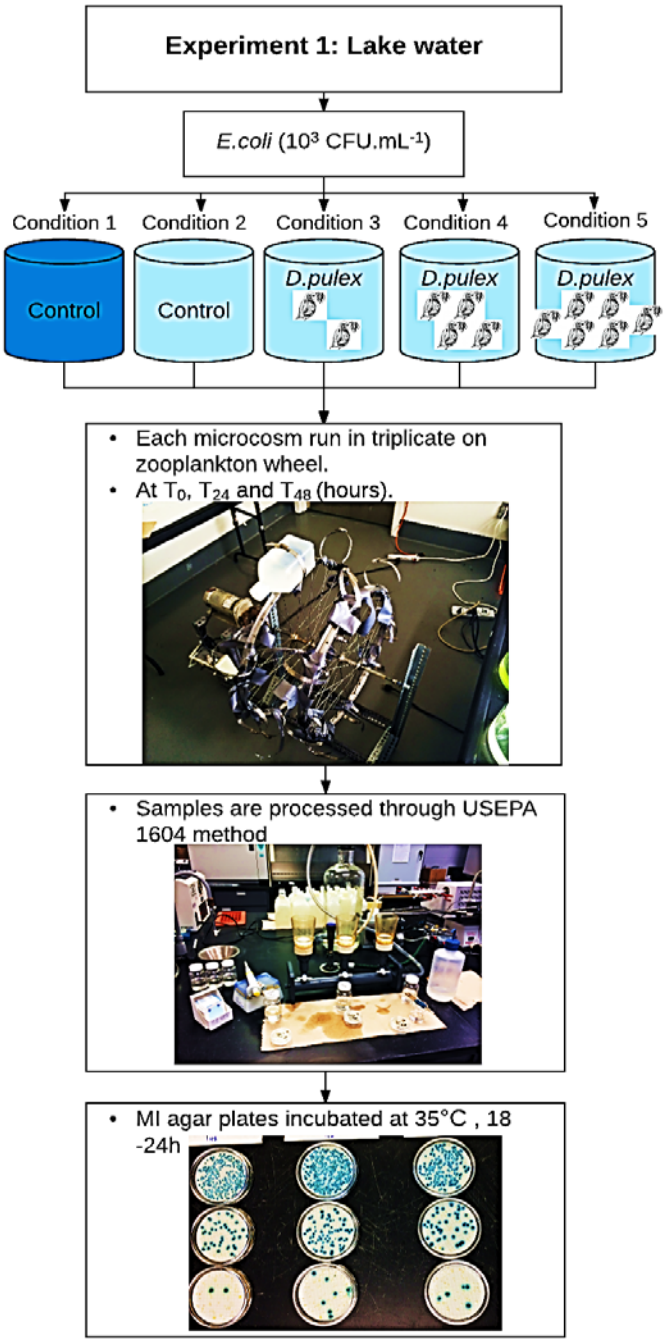

Figure 3. 10: Overview of the second experimental setup using lake water. Blue: raw lake water; light blue: 53 µm-filtered lake water.

To determine *E. coli* decay rates in a natural water matrix, lake water was collected from Missisquoi Bay, QC, Canada at the intake of the drinking water treatment plant (DWTP) in Philipsburg, on Sept 1, 2015 (Figure 3.10). The sample was passed on a 53 µm mesh-size filter to remove metazooplankton species (cladocerans, large rotifers and copepods). Triplicate bottle microcosms were run on the zooplankton wheel for 48 hours (*Daphnia* and control microcosms) with total volume of 1.3L. *Daphnia* microcosms were spiked with 10, 40, or 80 juveniles (~1 mm), for final densities of 8, 32, 65 ind.mL^{-1} respectively. Two control microcosms (absence of *Daphnia*) were run: one with unfiltered lake water, the other with 53 µm-filtered lake water (Table 3.2). The microcosms were run and processed the same way as described in 3.1 for the synthetic water medium (ADaM). Using *E. coli* counts, the decay rate was derived from Equation 2.

Table 3. 2: Experimental conditions for determining the fate of *E. coli* upon exposure to a gradient of *D. pulex* in lake water

Condition	*E. coli* (CFU/mL)	*Daphnia* densities (ind./L)	Lake water
1	10^3	0	Unfiltered
2	10^3	0	Filtered*
3	10^3	8	Filtered
4	10^3	32	Filtered
5	10^3	65	Filtered

*filtered on a 53 µm mesh size net

The lake water sample was also analysed for metazooplankton and protozooplankton communities. Zooplankton counts were carried out by Audrey Lafrenaye and Rémy Tadonléké for metazooplankton and for heterotrophic nanoflagellates (HNF), respectively (through a collaboration with Université de Montréal) and following described protocols (Tadonléké et al., 2005; Pinel-Alloul and Minoumi, 2015).

3.4 Impact of various metazooplankton species on *E. coli* decay rates

Impact of various species Two *Daphnia* species, *D. pulex* and *D. magna*, in addition to the rotifer *Brachionus calyciflorus* were incubated in microcosms with a 1400mL volume (river water and wastewater) and initial *E. coli* concentrations of 10^3 to assess their effect on the loss rate of *E. coli* (Figure 3.11). In order to take into account for the removal of *E. coli* from the water column through settling, the microcosm design was changed to beaker microcosms without any mixing during the incubation period.

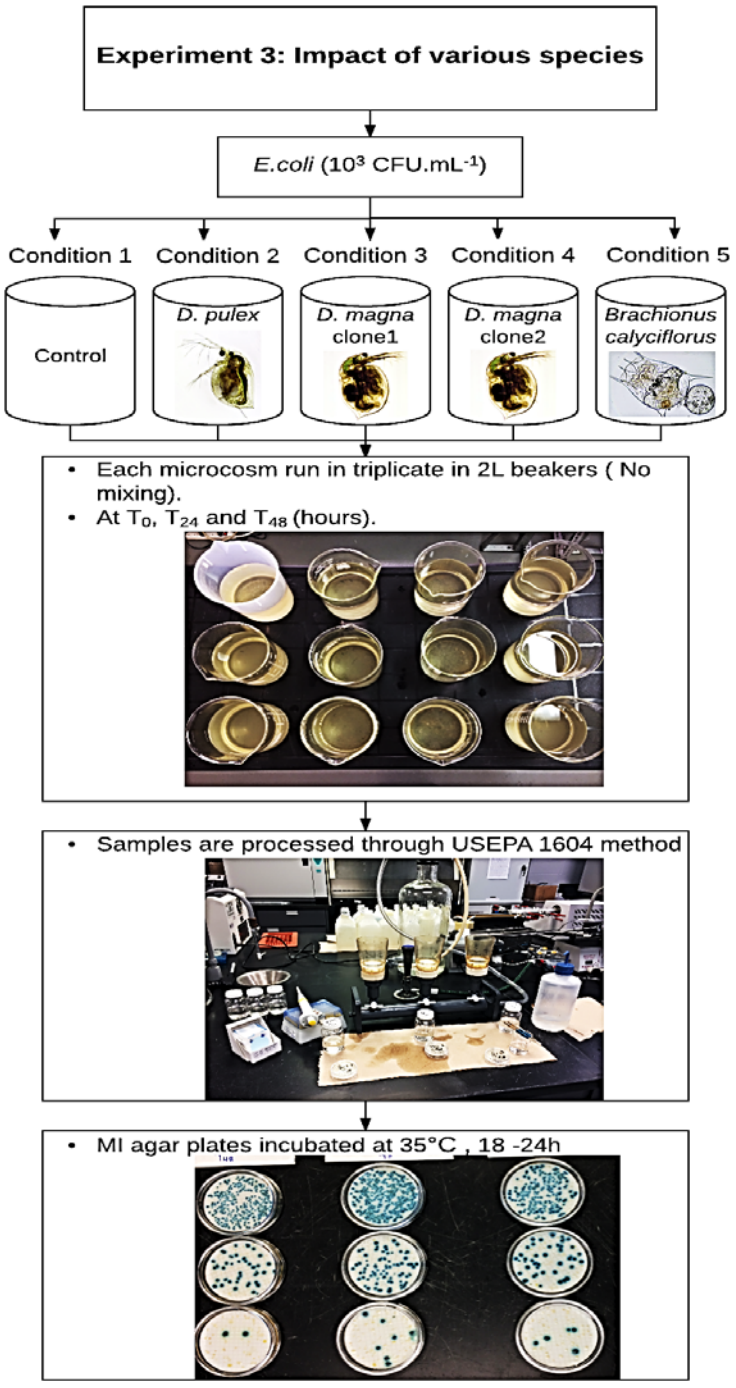

Figure 3. 11: Overview of the second experimental setup. *E. coli* with *D. pulex*, *D. magna* and *B. calyciflorus* in a matrix of river water mixed with primary wastewater (no mixing of the microcosms).

3.4.1 Preparation of microcosms

A surface water sample was collected from the Mille-Îles River at the intake of the Sainte-Rose drinking water treatment plant (DWTP) in Laval, QC, Canada and a primary effluent sample was collected from the Auteuil WWTP in Laval, QC, Canada between June and September 2016. Before starting the experiment, *E. coli* concentrations were determined in the primary effluent and river water samples using USEPA method 1604 (Figure 3.12).

On the following day, microcosms were prepared based on *E. coli* counts in primary effluent and river water and both samples mixed to reach a final concentration of *E. coli* of 10^3 CFU/mL using the following equation.

$$Ci \times Vi = Cf \times Vf \tag{3}$$

where; C_i is the initial concentration or concentration of stock solution, V_i is the initial volume or amount of stock solution needed, C_f is the final concentration or concentration of desired solution, and V_f is the final volume or volume of desired solution.

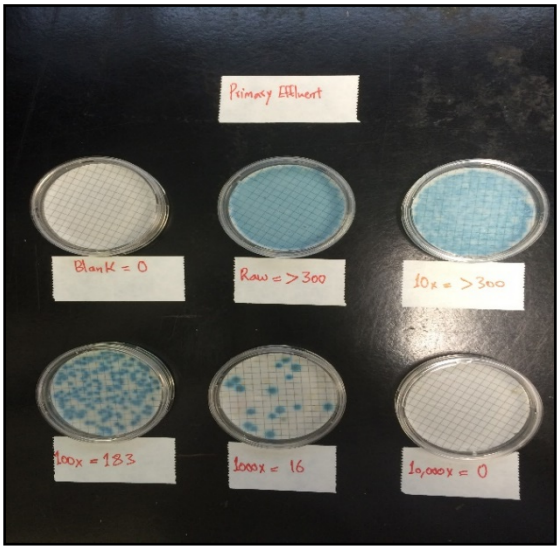

Figure 3. 12: Enumeration of *E. coli* in the primary effluent sample on MI agar plates (USEPA method 1604).

3.4.2 Experimental procedure

A pre-test was performed with *Daphnia pulex* only in order to test the new microcosm setup, which differed from the setup used in experiment 1 to account for any settling effects on the removal of *E. coli* from the water column.

Following the pretest, decay rates of *E. coli* were compared between *D. pulex*, *D. magna*, and the rotifer *Brachionus calyciflorus*. In addition to this, and given the fact that two different clones of *D. magna* were available at the Cristescu lab in McGill (clone1 "KLEINE" and clone2 "XINB3"), we decided to further assess whether or not predation pressures on *E. coli* could vary at a sub-species level for using both *D. magna* clones.

Two independent trials were performed. Because triplicate control microcosms were added to each trial, a maximum number of 12 microcosms could be processed at the same time (1 control and 3 zooplankton microcosms performed in triplicates each). Therefore, during the first trial, *E. coli* decay rates were compared only between *Daphnia* species and clones (i.e. *D. pulex* and both *D. magna* clones). In a second trial, *D. pulex* was compared to *D. magna* clone 2 (which caused a higher *E. coli* decay rate than clone 1) and to the rotifer *B. calyciflorus*.

Because a substantial natural *E. coli* removal already occurred in the control microcosms, *E. coli* decay rates were all expressed as *Daphnia*- or *Brachionus*-mediated decay rates (decay rates minus those measured in control microcosms).

Daphnia population densities were chosen based on the first experiment, which had shown that *Daphnia*-mediated decay rates of *E. coli* became significant above a *Daphnia* population density of 30 ind.L^{-1} (Section 4.1.2, figure Degans et al. (2002) also came to similar conclusions for .(4.4*Daphnia* grazing on natural bacterioplankton.

A total of 50 individuals were therefore added to the 1.4L beaker microcosms.

For the rotifer *Brachionus calyciflorus*, a total of approximately 700 individuals were added to the microcosms, resulting in population densities of ~500 ind.L^{-1} which corresponds to densities typically found in surface water (Table 3.3).

Table 3. 3: Concentrations of *E. coli* and zooplankton population densities used in experiment #2 to assess the differential impact of zooplankton species on the fate of *E. coli* in surface water microcosms spiked with raw sewage.

*E. coli** (CFU/mL)	Zooplankton species	Population densities (ind./L)
10^3	*D. pulex*	36
10^3	*D. magna clone 1*	36
10^3	*D. magna clone 2*	36
10^3	*Brachionus calyciflorus*	500

**E. coli* from raw sewage

The two assays were all run in the same ambient conditions as for experiment 1, at the same *E. coli* concentration (10^3 CFU.mL^{-1}) and under the same grazing pressure (36 individuals. mL^{-1}). *Brachionus calyciflorus* were added at concentration of around 500 individuals/mL^{-1}. All microcosms were run at 20°C ±1, during 18:6 light-dark cycles. Physical and chemical measurements (pH, oxygen, turbidity, conductivity, and temperature) were performed daily to monitor the overall evolution of the matrix using a HACH multi-parameter probe (HQ40d) (Figure 3.13).

For *E. coli* enumeration, samples were taken from the upper third of each microcosm to analyze at T_0, T_{24}, T_{48}. following USEPA method 1604 as described in section 3.6. At the beginning and at the end of the assay, total suspended solids (TSS) were analysed using, 500mL on a 1.2µm filter (GF/C glass microfiber filter, Whatman) preliminary dried at 105°C during >24h and weighed. After filtration of the sample, the filters were dried at 105°C during >24.and weighed to determine the concentration in TSS expressed in mg/L^{-1}. Because it was not possible to perform the analysis at T_{48}, the assays were run for another 24h and TSS analyses performed at T_{72}.

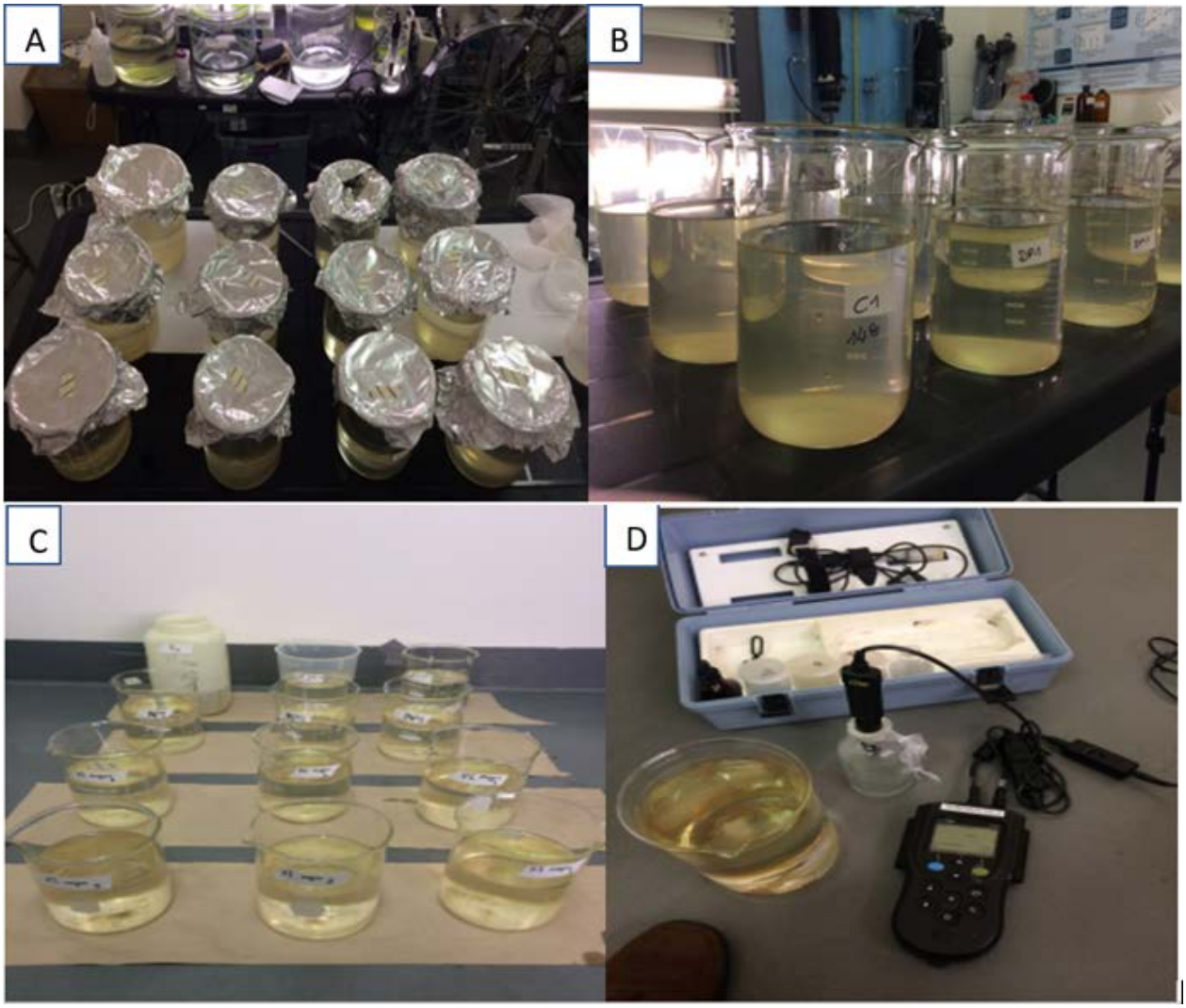

Figure 3. 13: Second experiment's microcosms, *E. coli* in presence or absence of *D. pulex*, *D. magna* (clone 1 and 2) and rotifers *B. calyciflorus*, in mixed river-raw sewage (A, B and C). Also, Physical and chemical measurements by HACH Probe (HQ40d) (D).

3.4.3 Enumeration of settled and *Daphnia*-associated *E. coli*

In order to assess the extent of *E. coli* removal from the water column through settling, bottom sediments were carefully collected with plastic pipettes at the end of the assay for control, *D. pulex* and *D. magna* microcosms. After dilution of the sample in phosphate buffer solution *E. coli* was enumerated using USEPA method 1604 (Section 3.6).

The portion of *E. coli* associated with *Daphnia* individuals was further investigated at the end of the second assay. Live *Daphnia* from each microcosm were collected with a plastic pipette and sonicated to lyse the bodies and release any associated and/or ingested *E. coli*. Then samples were filtered and cultured following USEPA method 1604 (Section 3.6).

Analysis of sediments and *Daphnia*-associated *E. coli* could not be done at T_{48} and had therefore to be done at T_{72}.

3.5 Impact of natural zooplankton communities on the decay rates of *E. coli* in lake water

3.5.1 Adaptation of the dilution method

The dilution method was initially developed to assess the grazing dynamics of marine zoo and phytoplankton communities (Landry and Hassett, 1980) and the method was adapted by Boehm et al. (2005) to study the impact of coastal zooplankton on the fate of *Enterococcus.* Here, we used the method to assess the impact of freshwater zooplankton communities (including metazooplankton and microzooplankton) on the fate of *E. coli* in lake water (Missisquoi Bay, QC, Canada).

The dilution method is based on the following assumptions:

- (1) growth or death of spiked bacteria is not influenced by the presence or absence of other spiked bacteria
- (2) the probability of spiked bacteria being consumed is a direct function of grazer encounter with the bacteria, and does not depend on the nutritional state of the prey. This is equivalent to assuming that the grazers are not food-satiated, and their consumption increases linearly with prey density.
- (3) $C(t) = C_0 \exp(-k-g)\, t$, where C is the concentration of spiked bacteria as a function of time t, C_0 is the concentration of prey at $t = 0$, k is the bacteria growth or inactivation rate and g is the rate of mortality due to ingestion by all size classes of grazers.

By diluting the collected lake sample with 0.22µm filtered lake water (at different ratios of unfiltered to filtered lake water), one dilutes the effect of grazing by the same percentage. Each sample of the dilution series is spiked with a known quantity of *E. coli* (1000 $CFU.mL^{-1}$) and incubation is performed during 24 hours. The apparent rate of change (d^{-1}) of *E. coli* concentration ($1/t \ln(C(t)/C_0)$) is determined by quantifying bacteria concentration at T_0 (C_0) and at T_{24} ($C(t)$), i.e. before and after the 24-h incubation period.

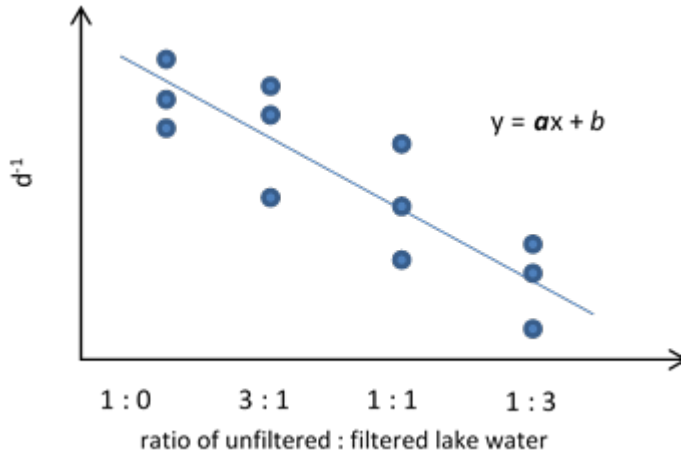

Figure 3. 14 : Grazing rates (*g*) correspond to the negative slope (*a*) of the line after linear-regression of the data (see figure above).

3.5.2 Sample collection and *in situ* analyses

Samples were collected during the zooplankton developing season (May/June to late October 2015) in the Venise-en-Québec bay area (see map figure 3.6, p.24). Sample collection aimed at covering the seasonal succession of zooplankton communities in terms of composition and relative abundance in Missisquoi Bay.

Samples were collected at 30cm below the surface and stored at 4°C before shipment to the laboratory. Additional samples (30L) were collected for zooplankton community characterization, and filtered *in situ* on a zooplankton net (53µm mesh size) to recover the metazooplankton fraction (cladocerans, rotifers and copepods). For preservation and later enumeration, the fraction was transferred into a 250mL bottle and anaesthetized with soda water before addition of a 4% formaldehyde solution (Figure 3.15).

For enumeration of flagellates and ciliates, two aliquots (250mL) of the filtrate (<53µm) were preserved either with 4% glutaraldehyde or with Lugol's solution, respectively (Figure 3.15).

In situ measurements (T°, pH, conductivity, DO, turbidity, chl *a* and phycocyanine) were performed using a multi-parameter probe (EXO 2, YSI, Inc.) (Figure 3.15).

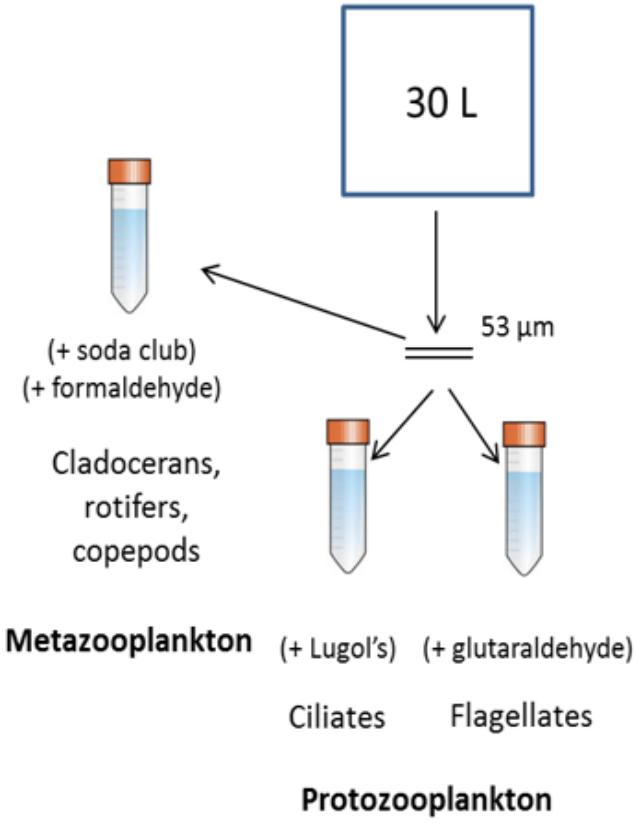

Figure 3.15: 30 L collected from Venise-en-Québec for zooplankton community characterization (53 µm mesh size).

3.5.3 Microcosm setup

Back to the laboratory, 10 L of the filtrate were sequentially passed on 1.2 µm and 0.22 µm nitrocellulose membranes to obtain filtered lake water (FLW) for subsequent dilutions. Triplicate 1L bottles were filled with initial matrix (including all size classes of grazers) and increasing dilutions of it, before being incubated on a rotating wheel (intermittent mixing at ~1 rpm) during 24 hours at 20°C (Figure 3.16).

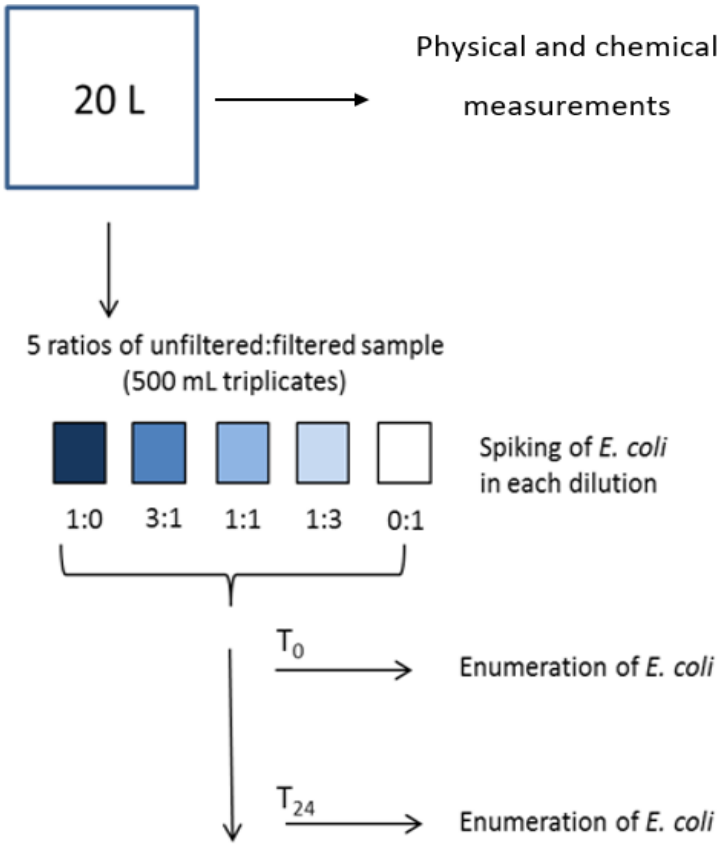

Figure 3.16: 20 L filtered on 1.2 µm and 0.22 µm nitrocellulose membranes to obtain filtered lake water for microcosms.

3.5.4 Laboratory analyses

Lake water samples preserved for metazooplankton and protozooplankton counts were analysed by Audrey Lafrenaye and Rémy Tadonléké for metazooplankton and for heterotrophic nanoflagellates (HNF) and ciliates, respectively (through a collaboration with Université de Montréal) and following described protocols (Tadonléké et al., 2005; Pinel-Alloul and Minoumi, 2015).

During the experiments, *E. coli* were enumerated in each microcosm at T_0, T_{24} and T_{48}, by culture following USEPA method 1604 (Figure 3.16).

3.6 Enumeration of *E. coli* USEPA method 1604

The membrane filter (MF) technique enumerates total coliforms on the surface of agar by providing a CFU/100mL count (APHA, 1985). By using the protocol of Method 1604 (U.S. Environmental Protection Agency, 2002), see appendix section 1.5.

The MI agar plates (BD Biosciences) were prepared and stored in the refrigerator at 4 °C (no more than 2-3 weeks). Once the samples were ready to be processed, we followed the following procedures:

a - Bunsen flame was turned on and the bottom of the MI agar plates was labeled with the sample number/identification and the volume of sample to be analyzed.

b - Placed the membrane filter (0.45µm pore size, 47mm diameter) on the porous plate of the filter base. Then, the funnel was attached to the base of the filter unit.

c - Samples were added to a 50mL sterile phosphate buffer to the filter unit. The vacuum was turned on and the funnel rinsed twice with sterile phosphate buffer.

d - The membrane filter was then removed from the filter unit and placed on the MI agar plate (gridside), then incubated over 18 - 24 hours at 35°C.

e - After the incubation, *E. coli* concentrations was evaluated by identifying and counting blue colonies under visible light.

3.7 Statistical analyses

One-way analyses of variance (ANOVA 1) followed by Tukey's *post hoc* test were performed to test the significance of differences in *E. coli* decay rates under the tested conditions. Linear regression was performed to analyse the relationship between culturable and viable *E. coli* cells as well as to verify that the decay rates followed a first order kinetic. All analyses were run in Statistica v.12 (StatSoft, Inc.). Significance was assessed at a $p<0.05$ level.

CHAPTER 4 RESULTS

4.1 Experiment 1 – Impact of *D. pulex* on *E. coli*.

4.1.1 Synthetic water

Daphnia pulex were incubated during 48 hours in the presence of *E. coli* in bottle microcosms containing synthetic water medium (ADaM). For the three conditions we tested, the decay rate of *E. coli* was significantly($p<0.05$) higher in presence of *D. pulex* than in its presence (Table 4.1). In the absence of *Daphnia*, concentrations of *E. coli* (10^6 or 10^3 CFU.mL^{-1}) remained rather stable over the duration of the experiment with average decay rates ranging between 0.05 and 0.12 d^{-1} (Figure 4.2). In the presence of *D. pulex* with *E. coli* spiked at 10^6 CFU/mL, the *E. coli* concentrations decreased from 10^6 to 2.3 10^4 CFU.mL^{-1} (Figure 4.1) representing a decay rate of 1.74 d^{-1}.

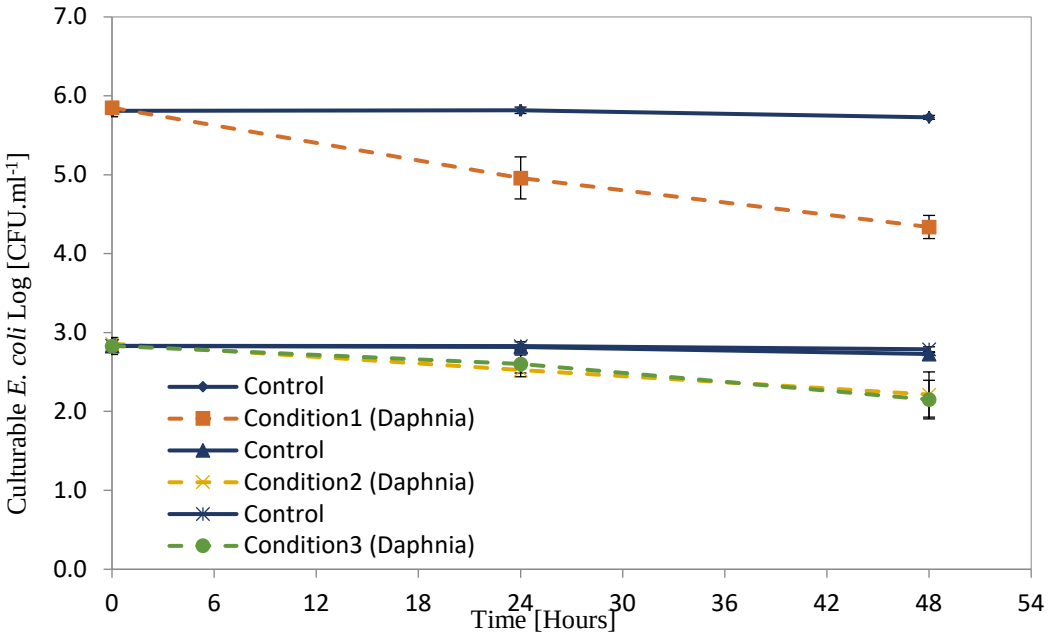

Figure 4. 1**:** Temporal evolution of *E. coli* concentrations in synthetic water (ADaM) in absence or presence of *Daphnia pulex* (32 ind. L^{-1}) during 48 hours and for varying *E. coli* (10^3 or 10^6 CFU.mL^{-1}) and algae (0.1 or 1.7 cells.mL^{-1}) concentrations. (*Daphnia* dashed lines, controls, full lines) 3 colors only, 3 conditions. See appendix section 2.1, table 1 for more details.

At lower *E. coli* concentrations (10^3 CFU.mL^{-1}, conditions 2 and 3), the *E. coli* concentrations dropped at a similar extent, irrespective to the algae amount, from 10^3 CFU. mL^{-1} to 1.9 10^2 and

1.6 10^2 CFU. mL^{-1} in presence of low or high algae concentrations (0.1 and 1.7 Cells.mL^{-1} respectively), thereby representing reaching decay rates of 0.74 and 0,78 d^{-1}, respectively (Figure 3.2). The same decay rates were observed in the presence of low and high algae.

Table 4. 1: First order kinetics of *E. coli* decay upon exposure to *Daphnia pulex*.

E. coli CFU/mL	*D. pulex* ind/L	*N. atomus* cell/mL	Equation	r	r2	p-value
10^6	32	6.6 10^3	y = 5,8 - 0,0319x	-0.98	0.96	p<0,00000
10^3	32	6.6 10^3	y = 2,8611 - 0,0132x	-0.88	0.77	p<0,01
10^3	32	1.3 10^5	y = 2,8778 - 0,0139x	-0.88	0.78	p<0,01

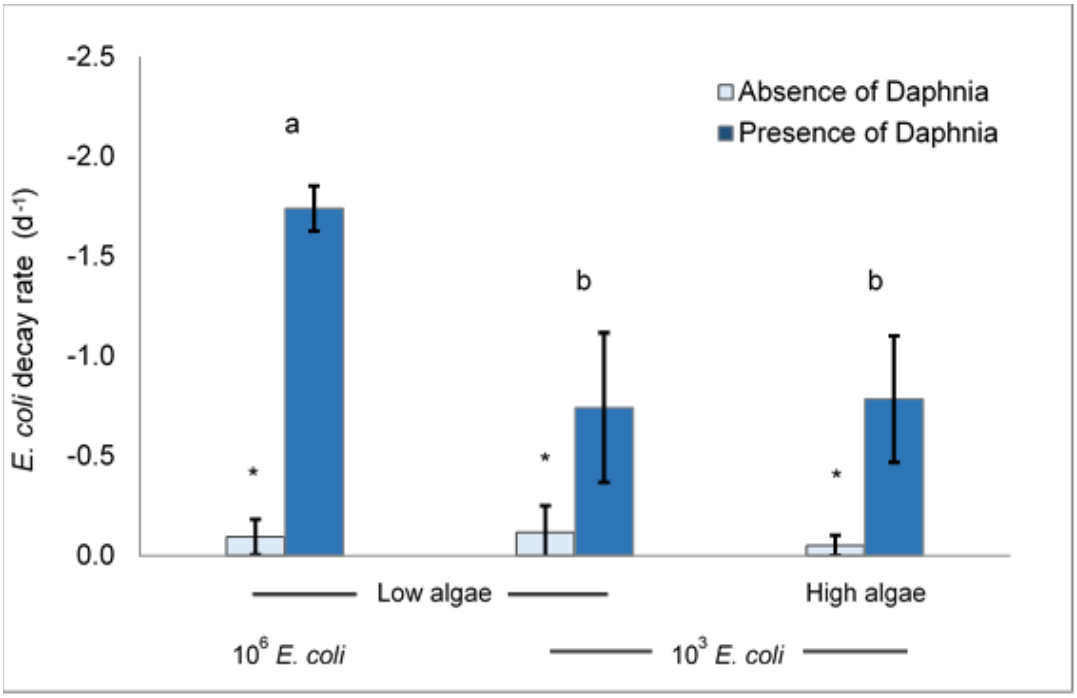

Figure 4. 2: Effect of *E. coli* initial concentration (10^3 or 10^6 CFU.mL^{-1}) and algae quantity (low or high algal food) on *E. coli* loss rates (in d^{-1}) following 48 hours incubation in absence or presence of *D. pulex* at densities of 32 ind.L^{-1}. Results from figure 4.1. were converted into decay rates using the formula described in page 25 (equation 2). Letters indicate significantly ($p<0.05$) different *E. coli* decay rates among conditions in presence of *Daphnia*. * significant ($p<0.05$) difference between absence and presence of *Daphnia* for a given condition (Burnet et al., 2017).

4.1.2 Lake water

Daphnia pulex (8, 32, and 65 ind.mL ^{1}) was incubated for 48 hours in presence of *E. coli* (10^3 CFU.mL^{-1}) in bottles containing filtered (< 53 µm) lake water. Two additional controls (absence of *Daphnia*) were run in triplicate in raw and filtered lake water in absence of *Daphnia* to measure the natural mortality of *E. coli*. In absence of *Daphnia* in raw lake water or filtered lake water, *E. coli* concentrations dropped by 0.8 and 1.0 Log (CFU.mL^{-1}), resulting in average decay rates of 0.92 and 1.15d^{-1}, respectively. No significant difference ($p > 0.05$) in *E. coli* decay rates was observed between both control conditions (Fig. 4.3). In presence of increasing *Daphnia* densities, *E. coli* decay rates were 1.04d^{-1} and 1.27d^{-1} and 1.62d^{-1} when exposed to 8, 32 and 65 ind.L^{-1}, respectively (Fig. 4.4). After subtraction of natural decay rates measured in filtered lake water, *Daphnia*-mediated *E. coli* removal rates were 0.12 and 0.47 for 32 and 65 ind.L^{-1}. No *Daphnia*-mediated removal of *E. coli* was observed in microcosms the lowest *D. pulex* densities 8 ind.L^{-1}; average decay rates of *E. coli* were even slightly lower than in filtered lake water devoid of *Daphnia* (Fig. 4.4).

The lake sample used in experiment 1 contained mainly rotifers (202 ind.L^{-1}) followed by small cladocerans (67 ind.L^{-1}) and copepod nauplii (55 ind.L^{-1}). No large cladocerans (*Daphnia sp*) were observed in the water sample. For protozooplankton, heterotrophic nanoflagellates occurred at population densities of 868 ind.mL^{-1} (Table 4.2). Ciliates were not counted in the lake sample collected for experiment 1.

Table 4. 2: Characterisation of zooplankton biota in the lake water matrix sampled at Missisquoi Bay (QC), Canada on Sept. 1, 2015. HNF, heterotrophic nanoflagellates; NA, not applicable; * expressed in ind.L^{-1} and ind.mL^{-1} for metazooplankton and protozooplankton, respectively; ** if >10 ind.L-1.

Zooplankton groups		Population density*	Dominant taxa**(≥10 ind.L^{-1})
Metazooplankton	Cladocerans	67	*Chydoridea sp, Bosmina longirostris, Ceriodaphnia sp*
	Rotifers	202	*Polyarthra sp, Keratella cochlearis,*
	Copepods	55	Nauplii
Protozooplankton	HNF	868	Not determined

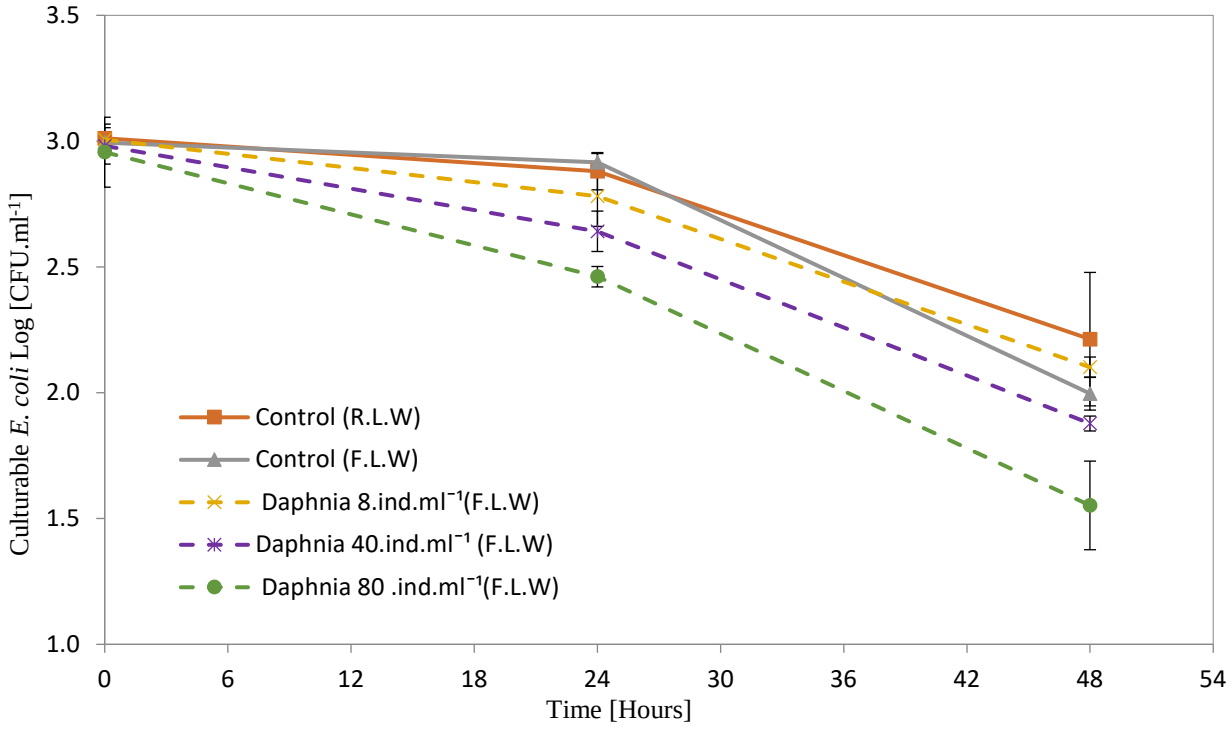

Figure 4. 3: Temporal evolution of *E. coli* concentration in lake water in presence of increasing *Daphnia pulex* population densities of 8, 32 and 65 ind. L^{-1}. Two controls, composed of raw and 53 µm-filtered lake water devoid of *D. pulex* were performed to assess the natural removal of *E. coli* in presence of local biota of all sizes and smaller than 53 µm, respectively. RLW, raw lake water; FLW, 53 µm-filtered lake water.

Table 4. 3: First order kinetics of *E. coli* decay upon exposure to *Daphnia pulex*.

E. coli CFU/mL	*D. pulex* ind/L	Type of matrices	Equation	r	r2	p-value
10^3	0	R.L.W*	y = 3,0889-0,0167x	-0.88	0.77	p<0.01
10^3	0	F.L.W*	y = 3,1333 - 0,0208x	-0.9	0.81	p<0.001
10^3	10	F.L.W	y = 3,0556 - 0,0181x	-0.93	0.97	p<0.001
10^3	40	F.L.W	y = 3,0389 - 0,0229x	-0.96	0.93	p<0.0001
10^3	80	F.L.W	y = 3,0333 - 0,0292x	-0.96	0.93	p<0.0001

*R.LW= Raw lake water, F.LW= Filtered lake water (53 µm)

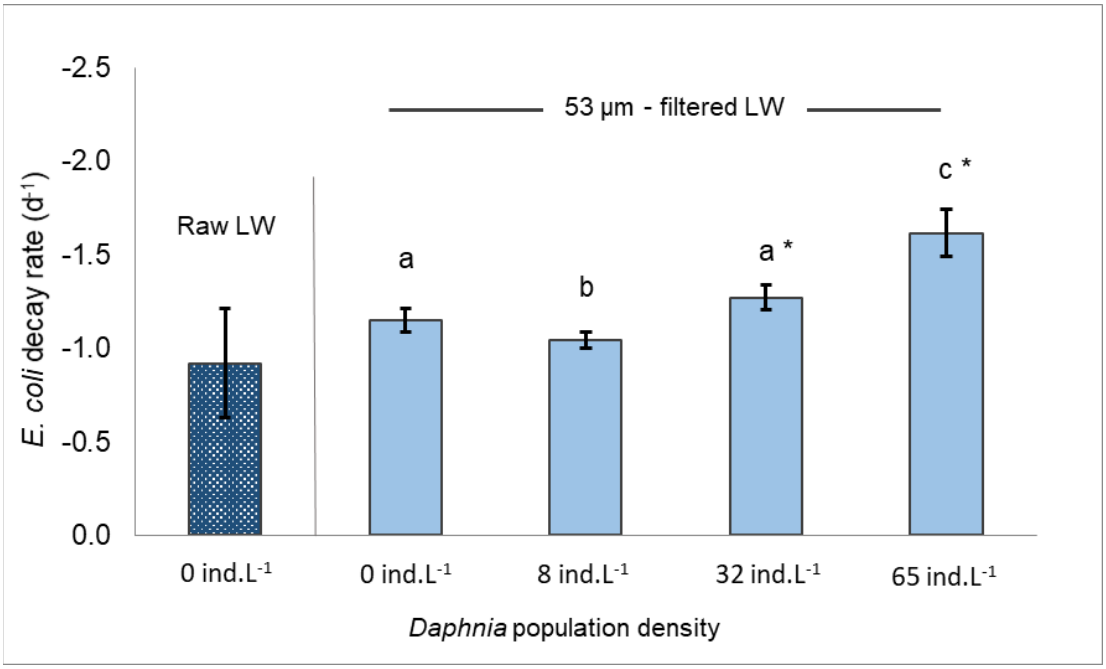

Figure 4. 4: Grazing of *D. pulex* on *E. coli* in a lake water matrix. Decay rates (in day^{-1}) of *E. coli* are measured following 48 hours incubation in presence of a *D. pulex* gradient (8, 32 and 65 $ind.L^{-1}$) in 53 µm-filtered lake water. Filtered or raw lake water samples were used as controls to determine *E. coli* decay rates in the absence of *D. pulex*. Letters indicate significant differences between treatments in filtered lake water; asterisks report significant differences from raw lake water. LW, lake water.

4.2 Experiment 2 – Impact of various metazooplankton species on *E.coli* decay rates

4.2.1 First assay

Daphnia pulex, *D. magna* clone 1 and *D. magna* clone 2 (36 ind.ml^{-1}) were incubated for 48 hours in the presence of *E. coli* (10^3 CFU.ml^{-1}) in 2L beakers containing river water mixed with raw sewage. Figure 4.5 and Table 4.6 show the decrease of *E. coli* concentrations over time when exposed to *Daphnia* spp.

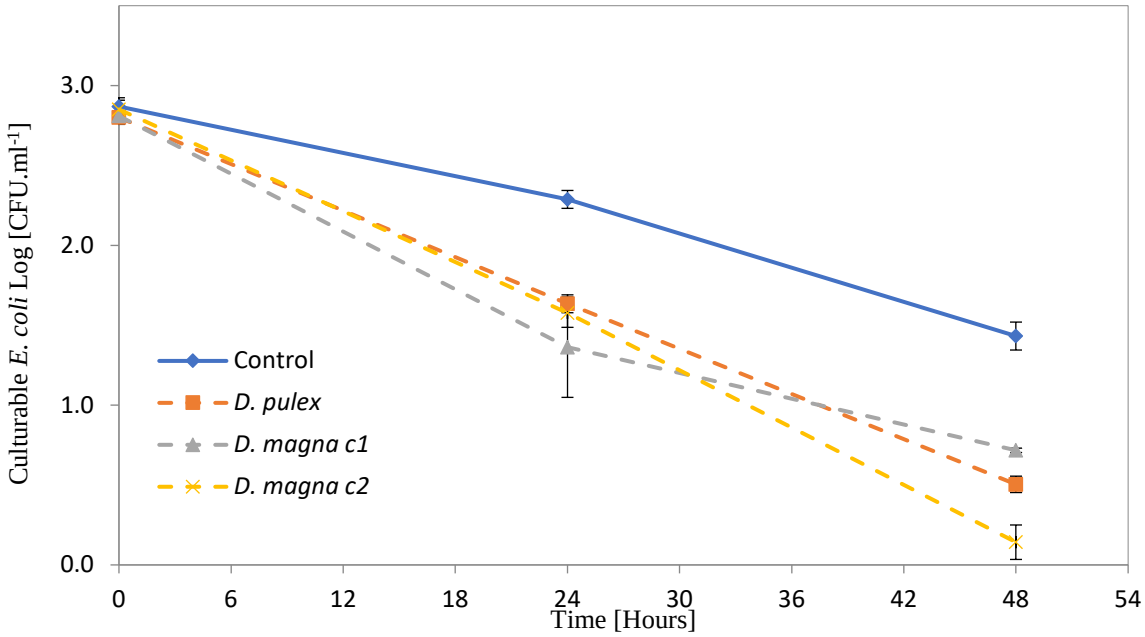

Figure 4. 5: *E. coli* concentration (10^3 CFU.mL^{-1}) versus time in water matrix (river water mixed with primary wastewater), in absence or presence of *Daphnia pulex* and *D. magna* (36 ind.L^{-1}) during 48 hours of incubation. The decrease in *E. coli* concentrations is more apparent with *Daphnia*.

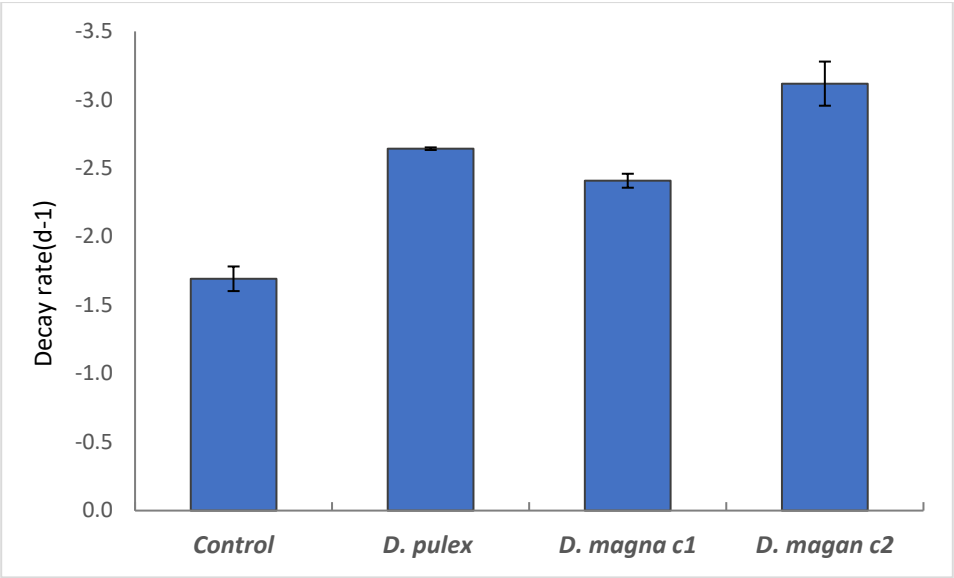

Figure 4. 6: *E. coli* average decay rates (in d^{-1}) following 48 hours incubation in the absence or presence of *Daphnia pulex* and *Daphnia magna* (clones c1 and c2) at densities of 36 ind.L^{-1}. *E. coli* were added to the microcosms at an initial concentration of ~10^3 CFU.mL^{-1}.

As shown in figure 4.6, the decay of *E. coli* in absence of *Daphnia* reached already 3.12 d^{-1} (1.43 d^{-1} after subtraction of the decay rates observed in control microcosms). In presence of *Daphnia*, *E. coli* decay rates were 2.64 d^{-1}, 2.41 d^{-1} and 3.12 d^{-1} for *D. pulex*, *D. magna* clone 1 and *D magna* clone 2, respectively. After subtraction of the decay rates measured in the control microcosms, and with corrected decay rates they were 0.72 d^{-1}, 0.99 d^{-1} and 1.43 d^{-1}, respectively (Figure 4.6).

4.2.2 Second assay

Following incubation of *E. coli* with *D. pulex*, *D. magna* and *Brachionus calyciflorus* for 48 hours, *E. coli* was influenced by *Daphnia magna* more than *Daphnia pulex* for identical population densities (36 ind.ml^{-1}). At population densities of 500 ind.ml^{-1}, *B. calyciflorus* did not exert a major grazing pressure on *E. coli* after 48 hours. In the absence of metazooplankton in control conditions, the decay rate of *E. coli* was almost negligible (0.14 d^{-1}), compared to the first assay (1.69 d^{-1}). In the presence of grazers, the decay rates of *E. coli* in were 0.76 d^{-1}, 2.48 d^{-1} and 0.35 d^{-1} for *D. pulex*, *D. magna* c1 and the rotifer *B. calyciflorus*, respectively. After subtraction of the decay rates observed in control microcosms, they were 0.62 d^{-1}, 2.33 d^{-1} and 0.21 d^{-1}, respectively (Figure 4.8).

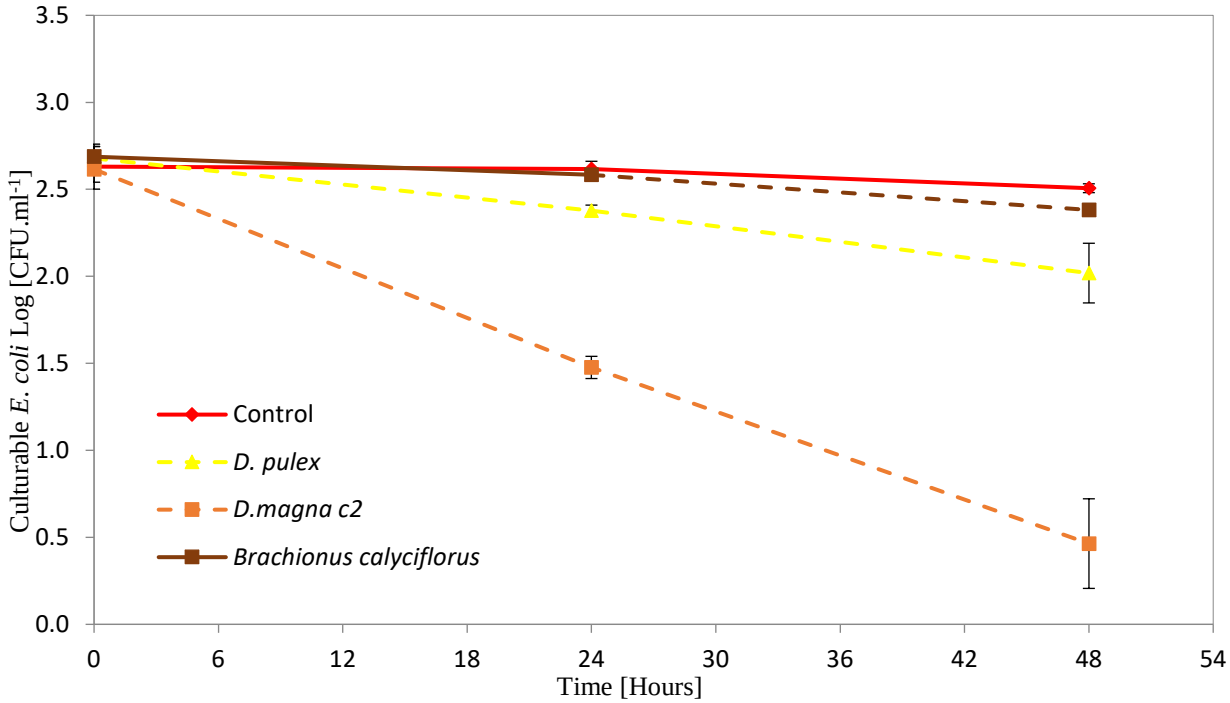

Figure 4. 7: Grazing of *D. magna* clone1, *D. pulex* and the rotifer *B. calyciflorus* on *E. coli* in a matrix of river water and primary wastewater effluent.

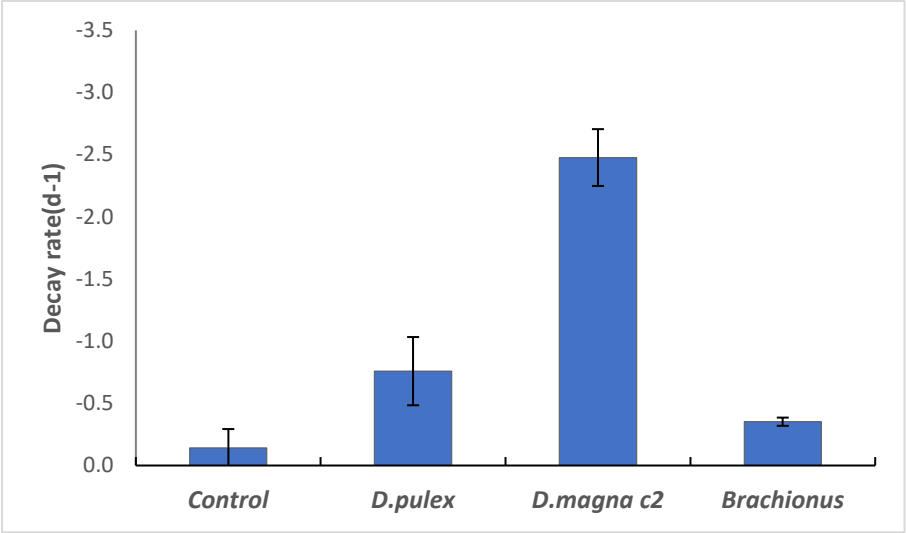

Figure 4. 8: *E. coli* decay rates (in d^{-1}) following 48 hours incubation in the absence or presence of *Daphnia pulex* and *Daphnia magna* (clone 2) at densities of 36 ind.L^{-1} and the rotifer *B. calyciflorus* at densities of 500 ind.mL^{-1}. *E. coli* were added to the microcosms at an initial concentration of ~10^3 CFU.mL^{-1}.

When combining the two assays, *D. pulex*-mediated decay rates varied between both assays, but they were still significantly ($p<0.05$) lower than those measured for *D. magna* clone 2. Decay rates induced by the rotifer *B. calcyciflorus* were significantly lower than those measured for Daphnia sp. (Figure 4.9).

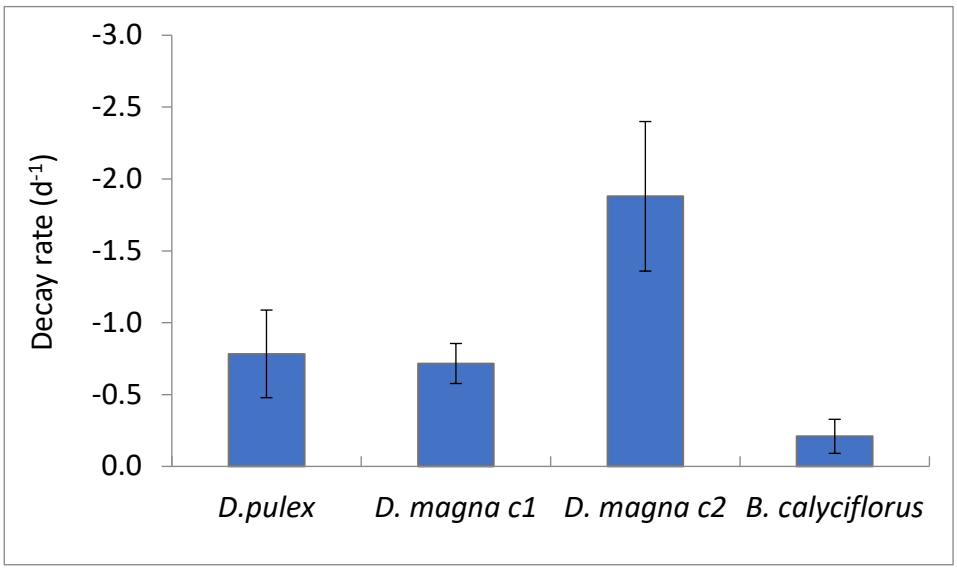

Figure 4. 9: Zooplankton-mediated decay rates (after subtraction of decay rates observed in control microcosms) for *D. pulex* (n=6), *D. magna* clone 1 (n=3), *D. magna* clone 2 (n=6) and *B. calyciflorus* (n=3).

4.2.3 Enumeration of settled and *Daphnia*-associated *E. coli*

At the end of the second assay (section 4.2.2), settled material was collected from a subset of microcosms and cultured ton MI plates (USEPA method 1604) in order to quantify any settled or excreted (but undigested) *E. coli*. In control microcosms, an average of 50 CFU.ml^{-1} were found *E. coli*, while in *D. magna* and *D. pulex* microcosms an average of 1 and 12 CFU.ml^{-1}was observed respectively (Figure 4.10 and 4.11).

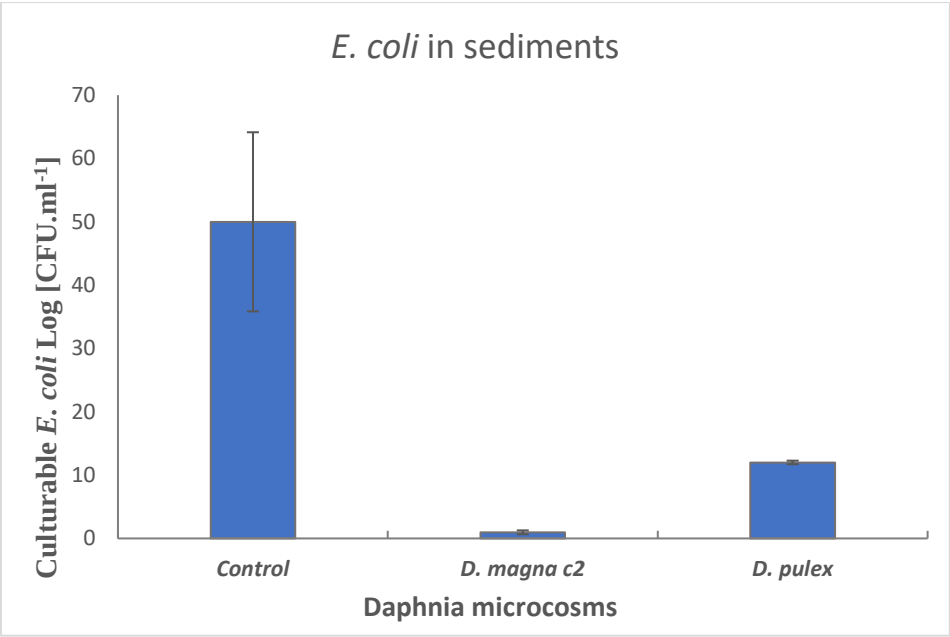

Figure 4. 10: Average concentrations of *E. coli* (CFU.ml^{-1}) remaining in microcosm sediments at the end of the second assay.

In *D. magna* microcosms, no *E. coli* associated to remaining individuals were reported by culture. In the *D. pulex* microcosms the *E. coli* count was 1 CFU.ml^{-1} (Table 4.4) (Figure 4.11).

Table 4. 4: The sonicated *Daphnia* and their *E. coli* results CFU.ml^{-1} at the end of the experiment.

Microcosms	Vol filtered (ml)	*E. coli* count (CFU.ml^{-1})
D. magna, microcosm 1	10	0
D. magna, microcosm 3	10	0
D. pulex, microcosm 1	19.4	1
D. pulex, microcosm 3	18.6	1

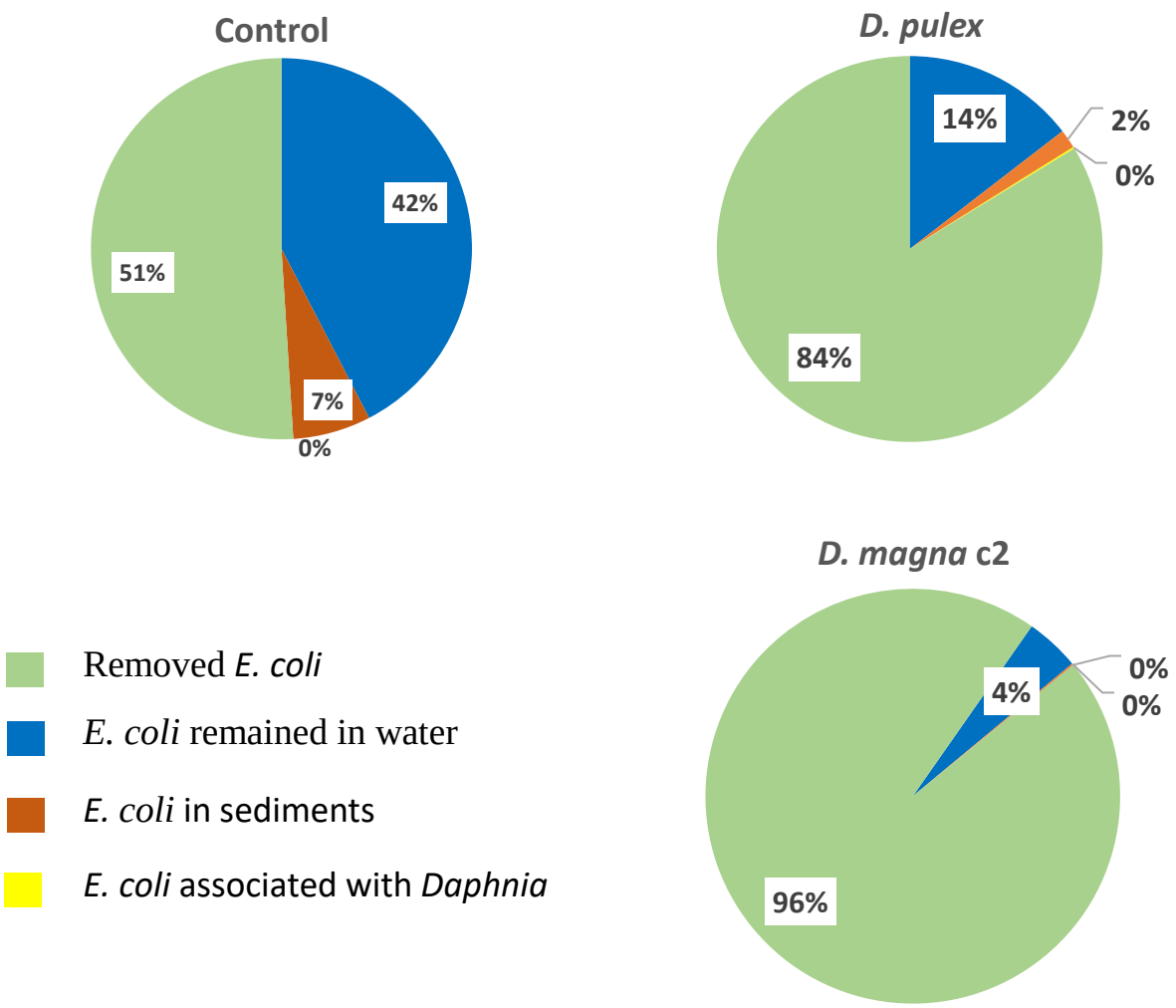

Figure 4. 11 : Illustrate the removed *E. coli*, remained in sediments, remained in water and associated with *Daphnia* at the end of experiment (second assay).

4.3 Impact of natural zooplankton communities on the decay rates of *E. coli* in lake water

Over the study season (May to October 2015), temperature, pH, dissolved oxygen and conductivity remained rater stable (Figure 4.12), except in October, where temperature dropped sharply to less than 10°C. Turbidity ranged between 2 and 9 NTU and was highest on July 7 and on September 23.

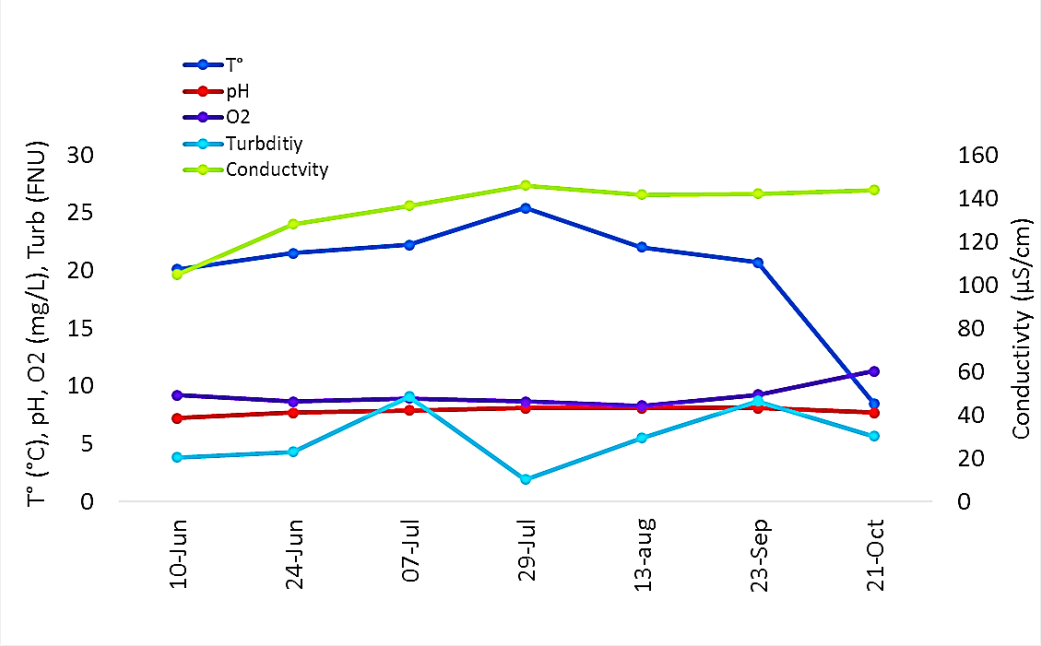

Figure 4. 12 : : *In situ* physical and chemical parameters for the 7 sampling occasions (June to October 2015).

The metazooplankton communities occurring in the samples collected from May to October 2015 were dominated by small rotifers. Copepods and Cladocerans were found in less densities, except in September and October (appendix section 2.4, Table A.10 in for details). The densities of metazooplankton groups also varied over the season and they increased in late June and in fall (Figure 4.13).

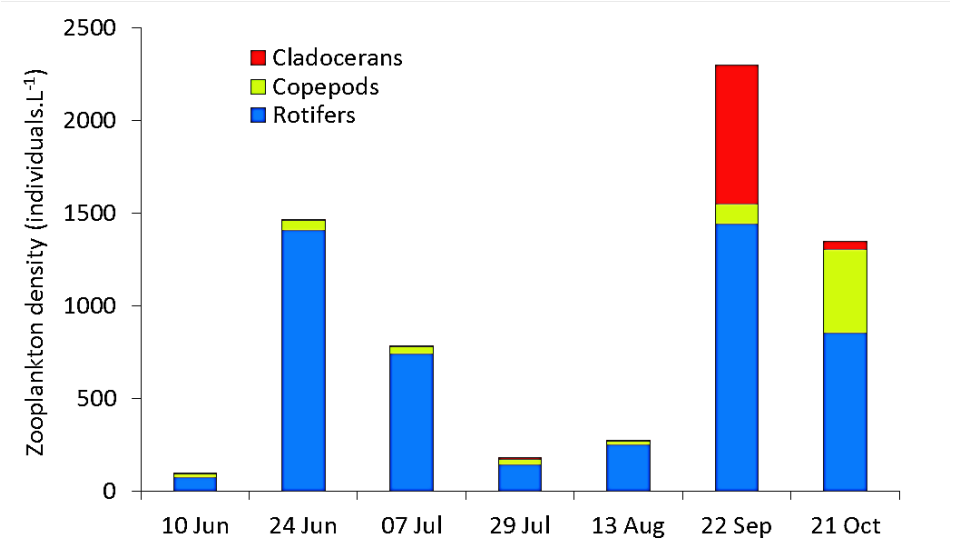

Figure 4. 13 : Metazooplankton relative densities (individuals.L^{-1}) for the 7 sampling occasions (June to October 2015).

The protozooplankton communities occurring in the samples collected from May to October 2015 were determined at group level, by counting the densities of heterotrophic nanoflagellates (HNF) and ciliates. For the June samples, no HNF counts were available. Marked variations in protozooplankton counts were observed over the season.

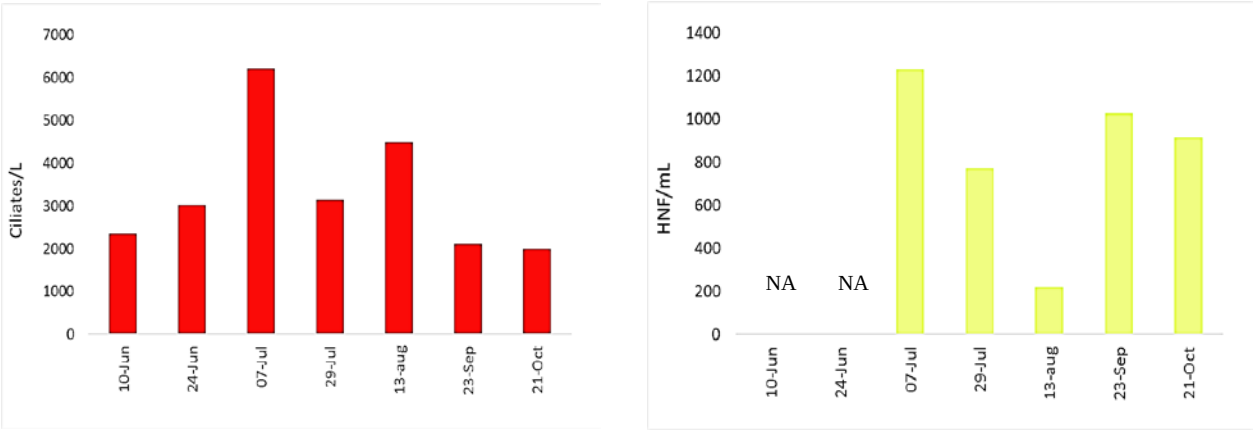

Figure 4. 14: Protozooplankton densities for the 7 sampling occasions (June to October 2015). Left: ciliates in individuals.L^{-1}; right: HNF in individuals.mL^{-1}. NA, not available.

Community grazing rates were found to vary also over the season and peaked on July 7 with a grazing rate of -0.8334. They were generally higher during the early summer than later in the season and dropped on October (Figure 4.15). It should be noted that all samples (*in situ* temperature between 20 and 25°C) were incubated at an ambient temperature of 20 ± 1°C, while the October sample was incubated at 8°C to reflect the *in situ* temperature.

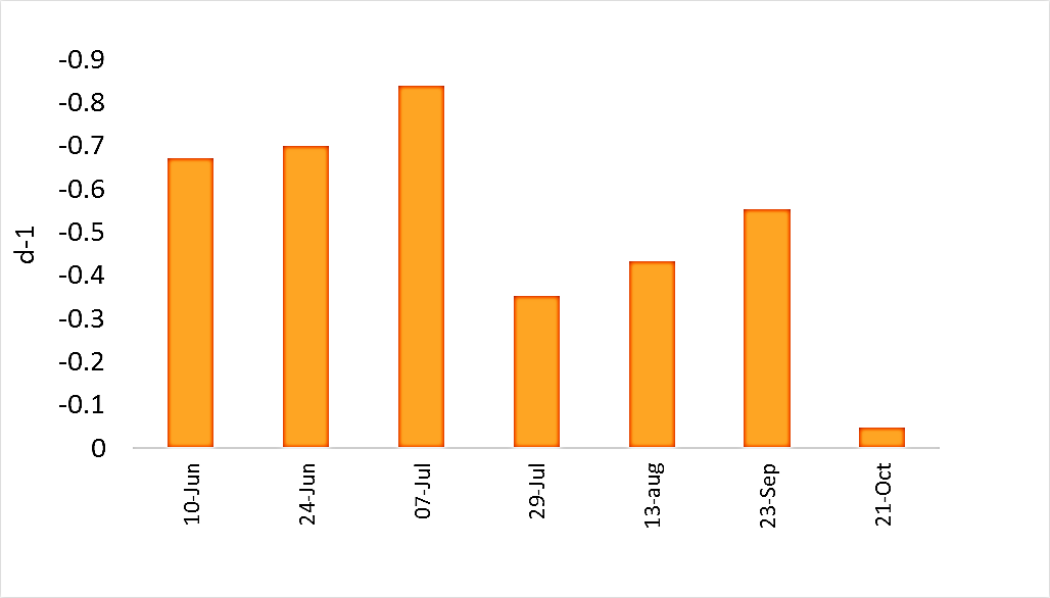

Figure 4. 15: Zooplankton community grazing rates (d^{-1}) as determined by the dilution method for the 7 sampling occasions (June to October 2015).

CHAPTER 5 DISCUSSION

5.1 Addressing research needs

The overall approach of the present study was to address the impact of zooplankton (in particular metazooplankton of the *Daphnia* genus) under various conditions by attempting to increase the representativeness of the exposure assays in view of the complex biotic interactions that are expected in the aquatic environment.

Exposure assays were first investigated in synthetic water matrices to compare with available data from the literature, and they were then designed to approach as much as possible natural conditions. As will be discussed later in this manuscript, additional improvements to our experimental approach can be made.

In a first set of experiments, microcosms are used to determine the impact of *Daphnia pulex*, an ubiquitous Cladoceran species, on the fate of culturable *E. coli* in synthetic water under a subset of laboratory conditions. Lake water is then used to take into account the role of indigenous organisms on the fate of *E. coli* when exposed to *D. pulex* in those environments,

Following exposure of *E. coli* to the model species *D. pulex*, the next step was to assess the impact of other common metazooplankton species. Concomitantly, *E. coli* spikes were performed using raw sewage instead of pure cultures in order to mimic the contamination of water resources on a drinking water resource and how metazooplankton could control this fecal pollution.

Finally given the fact the complex biotic interactions occur in aquatic environments and considering that protozooplankon can exert a substantial grazing pressure on microorganisms including *E. coli*, the overall impact of zooplankton on the fate of *E. coli* was assessed in surface water over several months in order to identify periods during which the local zooplankton biota would most affect the fate of *E. coli* in water.

In comparison to existing data on biotic interactions between planktonic filter-feeders and fecal microorganisms, which are particularly scarce to date, the present study provides new insights on those interactions and sheds light on the implications for the fate of *E coli* in aquatic environments. The present study focuses on *E. coli* as a prey for the filter feeding predators given the high

probability of encounter in water resources impacted by fecal pollution and the widespread use of *E. coli* as indicator of microbial water quality.

The following section discuss more in detail the results obtained for the different experiments performed during the present study.

5.2 Does *Daphnia* affect the fate of *E. coli* in water?

5.2.1 Synthetic water

During a first microcosm exposure experiment, a synthetic matrix was used, similarly to Schallenberg, et al. (2005) who assessed the effect of *Daphnia carinata* grazing on the survival of *Campylobacter*. After subtraction of natural losses obtained from control microcosms (1.4 Log_{10}) with an initial *E. coli* concentration of 10^6 (Condition 2), we observed a decay rate of $1.65d^{-1}$ that corresponded to a removal of 81% of the initial *E. coli* stock within 48 hours. These results are comparable to those of Schallenberg (2005), who found a 1.5–2 Log_{10} net removal after 48 hours of *Daphnia carinata* feeding on *Campylobacter jejuni*. Importantly though, we observed that only 54% of the initial *E. coli* stock was removed from the water by *D. pulex* when 1,000-fold lower initial *E. coli* concentrations were spiked in the microcosms (Figure 4.2). Feeding *Daphnia* with 1,000-fold lower *E. coli* concentrations led to decay rates of less than half ($0.62d^{-1}$), reflecting the lower probability of encounter between *Daphnia* and *E. coli* under these conditions. This is an important outcome as most feeding studies use very high concentrations ($\geq 10^6.mL^{-1}$) of fecal microorganisms (Connelly et al., 2007; Schallenberg et al., 2005; Fayer et al., 2000), which obviously tend to overestimate the impact of zooplankton grazing on the fate of these preys.

Using synthetic water, we assessed how the addition of algal food (which is the preferred food for *Daphnia*) would affect the loss rates of *E. coli*. The presence of low algal biomass (0.1 cells.mL^{-1}) or high algal biomass (1.7 cells.mL^{-1}) did not influence the average loss rates of *E. coli*, which were very similar between both conditions (0.74 and 0.78 d^{-1}, respectively) (Figure 4.2). This was unexpected as we hypothesized that the presence of algae would either reduce or increase (depending on their concentrations) the concomitant uptake of *E. coli*, as food selectivity by *Daphnia* is mainly based on particle size (Burns, 1968). Nevertheless, our findings were similar to those of Tezuka (1971), who showed that *Daphnia longispina* feeding rate on bacteria was not affected by the presence of algae. Although additional investigations should be attempted to

understand the effect of algae on the removal of *E. coli* by *Daphnia*, we can hypothesise that the similarity of *E. coli* loss rates in presence of low and high algal food amounts may to be due to a combination of feeding behavior and resistance of *E. coli* to gut passage. The low algae amount (0.1 cells.mL^{-1}) most probably forced *D. pulex* to decrease filtration efforts in order to save energy and thus ingest fewer bacteria over time. On the other hand, the high algae amount, which was preserved throughout the experiment at 1.7 cells.mL^{-1} could have provided adequate food for *Daphnia* to continuously ingest *E. coli*. As a result of the high nutritional value of algae as compared to *E. coli* bacteria (Freese and Martin-Creuzburg, 2013), the ingestion of *E. coli* may have been suboptimal. Culturability of *E. coli* may then have been preserved upon gut passage. The large discordance between observed and theoretical *E. coli* removal that we calculated using algal counts supports this assumption. An average of 6.6 ± 1.2 10^7 algae.d^{-1} were removed by *D. pulex* from each microcosm. This amount corresponds to an ingested volume of 0.7 ± 0.1mL. ind^{-1}.hour^{-1}, which is in the range of typical filtration rates for *D. pulex* (Jürgens, 1994). Thus, during 48 hours a total of 9.10^5 CFU would have theoretically been ingested by *D. pulex*, which means that the entire initial *E. coli* pool should have transited through the gut. Although our calculation is based on several assumptions (filtration rate was constant over time, ingested algal cells were all digested), it nevertheless suggests that *E. coli* culturability was maintained upon gut passage for at least part of the ingested *E. coli* population. Resistance of indigestible or low-quality food cells to gut passage in *Daphnia* has been demonstrated for lake bacteria (King et al.,1991).

Following these first results in synthetic water without any other grazing organisms, we assessed the grazing of *Daphnia* in lake water containing indigenous biota and tested how *Daphnia* population density would affect the decay rate of *E. coli* in lake water.

5.2.2 Lake water

In aquatic environments, complex interactions occur among local biota including metazooplankton, protozooplankton, and bacterioplankton. Although the assessment of these complex interactions within the food web were beyond the scope of this study, the objective was to better calculate the real contribution of *Daphnia* to the decay of *E. coli* in surface water, given the strong influence of bacterivorous protozooplankton, which is known to contribute significantly to bacterial loss (Menon et al., 2003; Sanders et al.,1989; Thouvenot et al.,1999 and Simek et al.,1997).

As illustrated in Figure 4.4, *E. coli* displayed non-negligible natural loss rates (~1.0 d^{-1}) after 48 hours in lake water in absence of *D. pulex*. Metazooplankton populations of bay water were dominated by rotifers and small cladocerans (Table 4.2), however they had no or very little impact on *E. coli* since loss rates between raw and filtered (53-µm) lake water were similar ($p>0.05$) (Figure 4.4 and Table 4.3). It can be hypothesized that *E. coli* natural losses likely resulted from a combination of protist grazing, bacterial competition, nutrient scarcity and temperature (Blaustein et al., 2013; Wanjugi and Harwood, 2013; Wcislo and Chrost, 2000; Menon et al., 2003). Zooplankton biota in Missisquoi Bay water contained heterotrophic nanoflagellates (HNF) at similar densities as those found in other eutrophic freshwater lakes (Güde, 1988). The HNF can be considered as major predators of bacterioplankton (Güde, 1988; Simek et al., 1997) and protozooplankton (particularly HNF) have been shown to account for up to 90% of *E. coli* mortality in river water, with loss rates ranging between 0.2 and 0.8d^{-1} (Menon et al., 2003).

As shown in figure 4.4, *E. coli* decay increased with *Daphnia* population density and peaked at 1.6d^{-1} in the presence of 65 *D. pulex* ind.L^{-1}. Interestingly, in the presence of 8 *D. pulex* ind.L^{-1}, the *E. coli* loss rates were significantly lower than in the absence of *Daphnia*. This could be due to the removal of protozooplankton bacterivores by *Daphnia*, which in turn limited their predation pressure on *E. coli* (Degans et al., 2002). Simultaneously, *Daphnia* densities may have been too low to counterbalance a decrease in protozooplankton bacterivory. In the presence of 32 *D. pulex* ind.L^{-1}, *Daphnia* density was high enough to exert a significant predation pressure on both *E. coli* and protists. Likewise, as mentioned by Degans et al. (2002), *Daphnia magna* at densities of 30 ind.L^{-1} were able to control HNF and ciliates and therefore becoming the dominant bacterivore. Additionally, the non-linear increase of *E. coli* loss rate with *D. pulex* densities may be related to crowding effects, which are known to occur above 30 ind.L^{-1} (Lürling et al., 2003; Helgen,1987). We can support this hypothesis by the fact that in the presence of *D. pulex* at 8, 32 and 65 ind.L^{-1}, respectively, the individual *Daphnia* contribution to *E. coli* loss rates progressively decreased from 0.13 to 0.04 and 0.02 d^{-1}.ind^{-1} (decay rate divided by the number of individuals in the microcosm).

Although *Daphnia* densities tested in experiments 1 and 2 (synthetic and lake water, respectively) are representative of those found in natural freshwater systems, *Daphnia* populations can seasonally peak above 100 and even exceed 1,000 ind.L^{-1} (Davies, 1985; Kvam and Kleiven, 1995). For instance, it has been reported that in aerated sewage ponds, high densities can occur (>500

ind.L^{-1}) (Cauchie et al, 2000; Daborn et al, 1978). Despite the existence of negative crowding effects, it is thus expected that *Daphnia* will have a strong impact on *E. coli* in these water samples.

5.3 How do different metazooplankton species affect the fate of *E. coli*?

The above described experiments in synthetic and lake water focused on the model species *D. pulex*. Other common Cladoceran species that can be found in lakes and ponds include *D. magna*, *D. pulicaria, D. mendotae,* or *D. carinata*. Since *D. magna* is the largest *Daphnia* species and given that it displays higher filtration rates than *D. pulex* (Jürgens, 1994), we decided to compare both species. Two different clones of *D. magna* were available at the Cristescu lab at McGill, and both were compared to *D. pulex*. To test another cladoceran species, we collected *Moina* sp from a wastewater treatment plant. Unfortunately, however, it was impossible to maintain the cultures, in which populations crashed several times because of unknown reasons. It is possible that *Moina* did not cope long term with the transition from secondary treated wastewater basins to culture media. Finally, the rotifer, *B. calyciflorus* was also added to the comparison given the widespread occurrence of rotifers in surface waters.

Two independent experiments were carried out to compare the metazooplankton species in terms of their impact on the fate of *E. coli*. In the first experiment, only *Daphnia* species were compared. In the second experiment, the *D. magna* clone displaying the highest grazing pressure on *E. coli* was selected and compared to *D. pulex* and *B. calyciflorus*. As explained in the material and methods section, the assays were limited to a maximum number of 12 microcosms (4 conditions in triplicates) that could be studied in parallel, including triplicate control microcosms.

The first assay (Figure 4.6) showed that *D. magna* clone 2 caused the highest decay rate on *E. coli*, followed by *D. pulex* and *D. magna* clone 1 (ANOVA 1, $p<0.05$). During the second assay, *D. magna* clone 2 still had the highest impact on *E. coli* decay rate, and was similar to that observed during the first assay. In contrast, *D. pulex* had a much lower impact on *E. coli* decay rate than during the first assay (Table 5.1).

Table 5. 1: *E. coli* loss rate (d^{-1}) in absence or presence of *Daphnia* spp and rotifer.

E. coli loss rate (d^{-1})					
	Control	*D. magna* clone 1	*D. magna* clone 2	*D. pulex*	*Brachionus calyciflorus*
First assay	1.65	3.12	2.41	2.64	-
Second assay	0.14	-	2.48	0.76	0.35

As previously mentioned in the methods section (Experimental procedure), the size of *D. magna* and *D. pulex* ranged from between lengths of ~2.9 and ~2.1 mm, respectively. Therefore, we can assume that this difference in efficiency and high ingestion capability by *D. magna* may correlated with the animal's body size (Brendelberger, 1991). This assumption is supported by the ratio of filtering of *Daphnia* on bacteria or algae as measure of relative efficiency found to be a threefold range in efficiency for animals at the extremes of the body size range. Furthermore, the larger sized animals are able to maintain higher filtering efficiency (Peterson et al., 1978; Knoechel et al.,1986). Similarly, Brendelberger (1991) reported that larger size of the *D. magna* contributed to a more efficient removal of natural bacterioplankton, which may also apply for fecal bacteria. Additionally, the turbidity decreased from around 30 to 10 NTU in *D. pulex* and *D. magna* conditions. Beyond the first 48 hours, in conditions with *D. magna,* an additional 14% turbidity removal was observed in a 24-hour period (Physico-Chemical Measurements, appendix section 2.3.2, Table A.9). The turbidity removal is expected to be associated with the nearly 95% *E. coli* removal by *D. magna*. The filtration of wastewater by *D. magna* appeared to be efficient for the removal of suspended sludge particles in wastewater. For example, a concentration of 50 ind.L^{-1} of *D. magna* during a 12h hydraulic retention times removed more than 30% of the particles through filtration and it has been shown to be effective for *E. coli* removal (Serra et al., 2016). Serra 2014 concluded that in tertiary treatment for wastewater reuse, the filtration performance of *D. magna* was much efficient in inactivating *E. coli* than conventional tertiary treatments. Also, in terms of nutrition, *D. magna* growth when feeding on heterotrophic bacteria (*Flavobacterium* and *E. coli*), was more efficient by 80-50%, respectively compared to *D. magna* fed on pure algal diets (Freese and Martin-Creuzburg, 2013). Heterotrophic bacteria ingested by *D. magna* are a substantial part of their source of carbon (40%) (Onandia et al., 2015).

In the absence of *Daphnia* (control microcosms) in the first and second assays, the *E. coli* displayed a non-negligible natural loss rate compared to presence of *Daphnia* microcosms with ~1.65 and $0.14d^{-1}$, respectively (Figures 4.6 and 4.8) (Table 5.1). The loss rate may be due to protozoa predation (ciliates, flagellates, and amoebae), where they are the most common components in biological wastewater treatment (Madoni., 2011). Protozoa are able to feed on particulates, and suspended bacteria and play an important role in maintaining the density of dispersed bacterial populations (Madoni., 2011). Additionally, the protozoa are well known as predators of bacteria (Trout et al., 2002; Agasild and Nogee, 2015; Barcina et al.,1997) and ciliates are able to reduce the density of viable *E. coli* in wastewater treatment (Curds and Fey, 1969).

In the presence of *D. pulex*, the *E. coli* loss rate for the first and second assays were 2.64 and $0.76d^{-1}$, respectively (Figures 4.6 and 4.8). It is apparent that the *E. coli* loss rate is higher in first assay than in the second one. As we measured, the *D. pulex* body length in first and second assays was (~2.5 and 2.1mm, respectively). This could be a reason for different decay rates and efficiency of filtering, where it has been reported that *D. pulex* filtering rate increases with the increase of *D. pulex* size (Peterson.,1978). From Burns (1969) and their *D. pulex* (body length and filtering rate) which is similar to our *D. pulex*, we could calculate the total volume filtered for our individual *D. pulex* in both assays theoretically (4 and 2.5mL ind^{-1} h^{-1}, respectively). Then, we calculated the total volume filtered in 48 hours by 50 *D. pulex* per one microcosm for first and second assay (6000 and 9600 mL/ microcosm h^{-1}, respectively). The difference of filtering rate ratio (total volume filtered) between the two *D. pulex* is (1.6 mL) and the difference between *D. pulex* specific loss rate (2.64 and $0.76d^{-1}$) is ($1.5d^{-1}$), after subtracted from the control microcosm. Therefore, this could explain the difference of *E. coli* loss rates between *D. pulex* in the first and second assays, where the small *D. pulex* (~2.1mm) in the first assay filtered less volume than the large *D. pulex* (~2.5) in the second assay. The difference in size can be attributed to the fact that harvesting and re-culturing the algae (*Daphnia*'s food) for long time made it poor nutritionally, and malnutrition of *Daphnia*'s mothers has strong negative effects on offspring quality (Ogonowski et al., 2016). Therefore, the *Daphnia* and their offspring became weaker and smaller. It is also known that *Daphnia* body sizes progressively decrease over time when maintained artificially in cultures (Cristescu, *personal communication*).

In the condition of *Brachionus calyciflorus*, had virtually no impact on *E. coli* concentrations over time and the decay rates were low ($0.35d^{-1}$) and comparable to those measured in control

microcosms (Figure 4.8). Similarly, Pérez-Morales et al. (2014) compared cladocerans and rotifers and showed that in terms of filtration rate and quantity of consumed food (algae: *Microcystis aeruginosa* and *Scenedesmus acutus*), the rotifers consumed much less than the cladocerans. Also, Ooms-Wilms (1991), indicated that rotifers showed a low removal of cultured bacteria and particles in the same size range as bacteria. Additionally, whereas protozoa such as ciliates are present in wastewater (Madoni, 2011) and rotifer *Brachionus calyciflorus* can prey upon and negatively affect the ciliates, heterotrophic nanoflagellates (HNF) and autotrophic flagellates (AF) (Mohr and Adrian, 2002), it can also ingest the ciliate *Coleps sp* by (5.7 ciliates rotifer-1 h^{-1}) with a clearance rate of (30 µl $rotifer^{-1}$ h^{-1}). This can be a reason that the *Brachionus calyciflorus* fed more on other organisms in wastewater (such as ciliates) than *E. coli* bacteria, where the rotifers can feed on bacteria but they are not food selective (Arndt Hartmut, 1993). It should also be noted that a high mortality rate occurred in *Brachionus* microcosms, which is not explained but may be due to the sudden exposure of the rotifers to sewage contaminated surface water. Further trials are needed to confirm the observations with *Brachionus* but these preliminary results shed light on the real impact of small rotifers on the fate of fecal microorganisms. Experimental ingestion studies that use very high concentrations of microbial preys (and hence very low predator to prey ratios) are very likely to overestimate the impact rotifers in aquatics environments. This enables us to highlight the need for experimental designs that are closer to real settings, in order to be able to provide representative results on the real impact of local biota on the fate of fecal microorganisms.

5.4 How do natural meta – and protozooplankton communities affect the fate of *E. coli* in water and how does their predation pressure evolve seasonally?

Exposure assays using one species of predator are useful to assess the tight predator-prey interactions, but they are not representative of natural conditions, where complex interactions occur between the various biota. Therefore, the last experiment was dedicated to the assessment of grazing rates by entire zooplankton communities (metazooplankton and protozooplankton) on the fate of *E. coli* in lake water using the same microcosm design as in experiment 1.

As shown in figure 4.14, grazing rates on *E. coli* varied over the season (from May to October). Highest grazing rates were measured in the first part of the summer in June and July ($0.84 d^{-1}$ on July 7), while they tended to be lower after July. The grazing rate measured in October were remarkably low though. Yet, population densities in October were similar to those measured in late June. Also, the metazooplankton communities were more diversified than during summer and were in part composed of small Cladocerans, which are more efficient grazers than rotifers. Actually, the virtual absence of any grazing activity on *E. coli* in October is more likely attributable to the much lower water temperatures. Indeed, since microcosm incubation was performed as much as possible at temperatures found *in situ*, the October assay was performed at an ambient temperature of 8°C in a controlled room. Yet, during the six sampling occasions preceding October, *in situ* temperatures averaged 22 ± 2°C and these assays were consequently carried out in a controlled room at 20 ± 1°C. The effect of temperature on the filtering rate in *Daphnia* and other zooplankton species is well known (Burns, 1969). Therefore, despite the diversity richness and the abundance of metazooplankton in October, their grazing impact on *E. coli* was almost null. Inversely, grazing impacts in July 29, when *in situ* temperatures of the collected matrix were 25°C, it is not excluded that the overall grazing impact the community on *E. coli* may have been underestimated at incubation temperatures of 20 ±1°C. It is also interesting to note that, from July onwards, cyanobacteria became more abundant in the water matrices collected in Missisquoi Bay. Complex interactions can occur between grazers, cyanobacteria, and fecal bacteria, many of which still need to be understood. Yet, it should be investigated if the overall decrease in grazing rate in late July and August could not be related to the presence of higher cyanobacteria densities that could affect the fitness of grazers and consequently reduce their pressure on bacterioplankton such as *E. coli*.

Overall, our results provide original and new data on the impact of zooplankton grazing on a fecal microorganism in a bay that is known to be impacted by fecal pollution and expands the first observations of Boehm et al. (2005) on the grazing of estuarine zooplankton on *Enterococcus* to freshwater biota and *E. coli*. The limited number of observations though (n=7) currently limits the investigation of any links between zooplankton (including metazooplankton and protozooplankton) species composition and relative abundance and their grazing pressure on *E. coli*. Although the methodology is labor-intensive and time-consuming, future trials are warranted to expand our knowledge on the grazing impact of natural zooplankton communities on the fate of fecal microorganisms (indicators and pathogens), since this knowledge is paramount for the assessment

and modeling of their fate in the environment. Major knowledge gaps still exist in this area, as physical determinants of the fate of microorganisms have been extensively studied to date.

CHAPTER 6 CONCLUSION AND RECOMMENDATIONS

Grazing experiments of *Daphnia* spp. and rotifer (different concentrations) were carried out using a range of concentrations for different potential food (*E. coli* and algae); which allowed the elucidation of their effects on the fate and viability of *E. coli* bacteria in drinking and recreational water.

This study demonstrates that, *Daphnia* spp. (*pulex* and *magna*) significantly impacted the fate of *E. coli* in water. Indeed, the effect of *D. pulex* on *E. coli* loss rates in lake water increased with population densities and overcame natural *E. coli* losses at densities between 32 and 36 ind.L^{-1}. When comparing the grazing impact of different metazooplankton species on the fate of *E. coli*, we showed that *D. magna* could remove more efficiently *E. coli* from the water, even if intraspecies differences were highlighted here too. Two different clones of *D. magna* did achieve different grazing pressures on *E. coli*. Rotifers, such as the model species *Brachionus calyciflorus* did not appear to negatively impact the fate of *E. coli* at a population density to be expected in natural settings. Although the focus of the present study was metazooplankton, there is no denying that protists such as heterotrophic nanoflagellates or ciliates can exert a much higher grazing pressure on bacterioplankton than metazooplankton. The interactions between zooplankton groups and how these interactions affect the fate of bacteria was obviously beyond the scope of this study, which was aimed at identifying the conditions under which grazing rates on *E. coli* could increase, and ultimately should help understanding the role of zooplankton on the fate of the fecal indicator *E. coli*. Nevertheless, our results (experiments 1, 2 and 3) indicate clearly the role of naturally occurring biota on the mortality on *E. coli* in water. We therefore strongly recommend the following methodological points when addressing the role of zooplankton on the fate of fecal microorganisms:

- Keep spike concentrations of target microorganism as low as possible (given the analytical constraints for the detection of low amounts of target microbe during exposure assays)
- Test representative population densities of grazer in order to avoid overestimating their impact on the microorganism because of higher encounter probability

- Use natural water matrices when assessing the grazing dynamics and intensities of zooplankton on the fecal pathogen or indicator. This is important given the ubiquitous occurrence of protozooplankton in water and their potentially high(er) impact on the target microorganism than the predator under study.

We would like to highlight potential improvements on the methodology applied in the present study.

The major improvement than could be done in future investigations would be to augment the representativeness of our conclusions by using mesocosms instead of microcosms that still represent a simplified and more controlled view of the aquatic habitat.

Also, instead of using a pure culture for the dilution method, we could have used a similar spiking suspension as that used in experiment 2, in order to mimic the contamination of the lake water by an entire consortium of fecal bacteria including *E. coli*, as is observed during pollution events.

Finally, instead of using “calibrated” *Daphnia* populations with individuals of identical body size, it would be more pertinent to use mixed populations comprising newly hatched individuals, juveniles and adults. Using “calibrated” populations of mixed body sizes and comparing them under various conditions could hep understand the grazing of *Daphnia* under fluctuating population dynamics. These investigations may be more of ecological interest, although they could also prove helpful for studies on the use of *Daphnia* as sustainable tool for tertiary treatment of fecal contaminants.

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APPENDIX A – MATERIALS PREPARATION AND RAW RESULTS

1. Materials preparation

1.1 Synthetic water medium recipe (ADaM)

Recipe of ADaM, an artificial freshwater for the culture of zooplankton (Kluttgen et al., 1994). To produce a given amount of medium, add the amounts of sea salt and stock solutions as given in the following table (Table A.1). For sea salt, we bought it from an aquarium supply store.

Table A. 1 : The quantities of sea salt and stock solutions to prepare the ADaM medium.

Water	Sea salt [g]	Stock solution A [ml]	Stock solution B [ml]	Stock solution C [ml]
10 L	3.33	23	22	1
50 L	16.6	115	110	5
60 L	19.9	138	132	6

The stock solutions:

Stock solution	Chemical	Concentration [g/l]
A	$CaCl_2 \times 2H_2O$	117.6
B	$NaHCO_3$	25.2
C	SeO_2	0.07

1.2 EPA recipe

Moderately hard water (EPA medium) (Weber,1991) This medium is used to culture zooplankton. Which is dissolving 96 mg $NaHCO_3$, 60mg $CaSO_4.2H_2O$, 60mg $MgSO_4$, and 4 mg KCl in one liter of distilled water (Table A.2).

Table A. 2 : The added reagents and final water quality to prepare the EPA medium.

	Reagent Added (mg/L)[2]			Final Water Quality			
	$NaHCO_3$	$CaSO_4.2H_2O$	$MgSO_4$	KCl	pH[3]	Hardness[4]	Alkalinity[4]
Very soft	12.0	7.5	7.5	0.5	6.4-6.8	10-13	10-13
Soft	48.0	30.0	30.0	2.0	7.2-7.6	40-48	30-35
Moderately Hard	96.0	60.0	60.0	4.0	7.4-7.8	80-100	60-70
Hard	192.0	120.0	120.0	8.0	7.6-8.0	160-180	110-120
Very hard	384.0	240.0	240.0	16.0	8.0-8.4	280-320	225-245

1.3 BBM recipe

Modified BBM (Bold's basal medium) is a freshwater algae medium that has been used to grow a variety of green algal cultures (Brown et al., 1964; Nichols and Bold, 1965). This medium was used to maintain our algae culture. The recipe was kindly provided by the University of Liège (Belgium).

Table A. 3 : The components of stock solutions, trace elements and vitamins to prepare the BBM medium.

Components	Concentration of stock solutions (g/500 mL)	Volume to add per liter (mL)	Final concentration in the medium (mg/L)
KNO_3	12,5	10	250
$CaCl_2$. $2H_2O$	1,25	10	25
$MgSO_4$. $7H_2O$	3,75	10	75
K_2HPO_4. $3H_2O$	3,75	10	75
NaH_2PO_4	8,75	10	175
NaCl	1,25	10	25
Trace elements	See below	2	-
Vitamins	See below	2	-
H_2O MilliQ	-	936	-

Trace elements:

Components	Concentration of stock solutions (g or mg/500 mL)
NaFeEDTA	2,5 g
$MnCl_2$.$4H_2O$	90 mg
$CuSO_4$. $5H_2O$	5 mg
$ZnSO_4$. $7H_2O$	11 mg
$CoCl_2$. 6 H_2O	5 mg
Na2MoO4. $2H_2O$	3,2 mg

. Vitamins:

Components	Concentration of stock solutions (mg/500 mL)
Thiamine hydrochloride	100
Biotin	5
Vitamin B12	5

1.4 The protocol for routine maintenance of the algal culture

For maintenance of the algal culture, the steps are performed per the following:

a). Preparation of stock solutions (every 2-3 months)

· Weigh each component and prepare 500-mL stock solutions (tables above)

· In a glass bottle, dissolve each salt in MiliQ water (by gentle manual mixing)

· For vitamins, wrap the entire bottle with aluminum foil to protect from light exposure

· Store at 4°C until use for the preparation of fresh BBM medium.

b). Preparation of fresh BBM medium (liquid) for sub-culturing the algae.

- For preparation of 500 mL of liquid BBM:

· Add 468 mL of MilliQ water into a 1-L Erlenmeyer flask

· Add 5 mL of each of the 6-salt stock solution

· Add 1 mL of trace metal stock solution

· Mix gently manually

· Add a stir bar (should be large enough for 500 mL to be mixed thoroughly)

· Add the stopper/glass tube device but do not close the flask hermetically. For this purpose, place a folded piece of gauze between the bottleneck and the stopper.

· Cover the stopper/neck as well as the glass tubing with aluminium foil

· Autoclave the flask at 121°C during 15 minutes

· After autoclave, let the medium cool down to ambient temperature (~20°C)

Add 1 ml of vitamin stock solution and mix gently.

1.5. Method 1604: Total Coliforms and *Escherichia coli* in Water by Membrane Filtration Using a Simultaneous Detection Technique (MI Medium)

Instructions for preparation of MI medium (U.S. Environmental Protection Agency, 2002).

1.5.1 Preparation

Media preparation:

Table A. 4 : The components of Dehydrated MI agar and deionized or distilled water for EPA medium preparation.

Ingredients	To make 100 mL	To make 1L
Dehydrated MI agar	3.65 g	36.5 g
Deionized or distilled water	100 mL	1000 mL

- Add amounts specified in above table of dehydrated MI agar to deionized or distilled water in the appropriate-sized flask.
- Stir this mixture for several minutes to break up clumps. Make sure that none of the medium adheres to the bottom or side of the flask.
- Place the flask in a heated water bath or on a hot plate and heat slowly to boiling. If using a hot plate, stir the mixture constantly or use a stir bar and magnetic stirring hot plate to prevent scorching. After boiling begins, remove the flask from the heat source.
- Aliquot 100-mL volumes into autoclavable bottles and autoclave at 121°C and 15 lb/in2 pressure for 15 minutes. Allow to cool to 45-50°C. Either pour plates (step 5) or store 100-mL aliquots of medium at 4°C (step 6).
- Pouring plates:
    - Preparation for pouring:
        - Prepare cefsulodin solution by adding 0.02 g of cefsulodin to 20 mL of deionized or distilled water. Sterilize the cefsulodin solution by filtering through a disposable, sterile 0.22-µm syringe filter. Store in a sterile

container at 4°C until needed. Prepare a fresh solution each time MI is made. Do not save any unused portion because it will degrade.

- Cefsulodin solution is added after the MI medium is autoclaved and cooled to 45-50°C.
- Add 0.5 mL of freshly prepared cefsulodin solution to each 100-mL volume of tempered agar medium and mix gently.

- Pour 6 to 7 mL of the medium into 50-mm Petri-dish bottoms. Quickly place the Petri-dish tops loosely onto the bottoms to allow condensation to escape.
- When the medium has solidified (about 10 minutes), close the Petri dishes by pressing firmly on the tops. These plates are suitable for use after the medium has solidified. About 15 to 20 plates can be prepared from 100 mL of medium. Label and date.
- Prepared Petri dishes, sealed in small plastic bags to prevent drying, can be stored in a refrigerator for up to 2 weeks.

- Long-term storage:
  - 100-mL aliquots of MI medium without cefsoludin can be stored at 4°C for up to 6 months.
  - To prepare plates from refrigerated agar, melt the medium using a beaker with water on a hot plate or by placing in the autoclave for a 5-minute cycle. Add cefsulodin solution as indicated in step 5.a and follow above instructions to pour plates.

1.5.2 Data Analysis and Calculations

- Use the following general rules to calculate the *E. coli* or TC per 100 mL of sample:
- 5.2.1.1 Select and count filters with ≤ 200 total colonies per plate.
- 5.2.1.2 Select and count filter with ≥ 100 target colonies (ideally, 20-80).
- 5.2.1.3 If the total number of colonies or TC on a filter are too-numerous-to-count or confluent, record the results as "TC^+ (TNTC)" and count the number of *E. coli*. If both target organisms are $ 200, record the results as "TC^+ EC^+ (TNTC)".
- 5.2.1.4 Calculate the final values using the formula:

$$\text{E. coli/100mL} = \frac{\text{Number of blue colonies}}{\text{Volume of sample filtered (mL)}} \times 100$$

$$\text{TC/100mL} = \frac{\text{Number of fluorescent colonies} + \text{Number of blue, non} - \text{fluorescent colonies (if any)}}{\text{Volume of sample filtered (mL)}} \times 100$$

- 4.2.6 See the USEPA Microbiology Manual, Part II, Section C, 3.5, for general counting rules.
- 4.2.7 Report results as *E. coli* or TC per 100 mL of drinking water.

2. Raw results

2.1 Synthetic water results

Table A. 5 : Temporal evolution of *E. coli* upon exposure to *D. pulex*. Results are shown as average ± standard deviation (n=3) andare expressed in CFU.mL^{-1} (log CFU.mL^{-1}).

Condition 1		
Time (hours)	Control microcosms	*Daphnia* microcosms
0	$6.8\ 10^5 \pm 1.4\ 10^5$ (5.8 ± 0.1)	$7.1\ 10^5 \pm 1.2\ 10^5$ (5.8 ± 0.1)
24	$6.6\ 10^5 \pm 6\ 10^5$ (5.8 ± 0.1)	$1\ 10^5 \pm 4.9\ 10^4$ (5 ± 0.1)
48	$5.4\ 10^5 \pm 3\ 104$ (5.7 ± 0.1)	$2.3\ 10^4 \pm 8.1\ 10^3$ (4.3 ± 0.1)

Condition 2		
Time (hours)	Control microcosms	*Daphnia* microcosms
0	$6.9\ 10^2 \pm 1.7\ 10^2$ (2.8 ± 0.1)	$7.3\ 10^2 \pm 9.3\ 10^1$ (2.9 ± 0.1)
24	$6.6\ 10^2 \pm 4.5\ 10^1$ (2.8 ± 0.1)	$3.4\ 10^2 \pm 7\ 10^1$ (2.5 ± 0.1)
48	$5.4\ 10^5 \pm 2\ 10^1$ (2.7 ± 0.1)	$1.9\ 10^2 \pm 9.5\ 10^1$ (2.2 ± 0.1)

Condition 3		
Time (hours)	Control microcosms	*Daphnia* microcosms
0	$6.8\ 10^2 \pm 4.6\ 10^1$ (2.8 ± 0.1)	$6.8\ 10^2 \pm 6.2\ 10^1$ (2.8 ± 0.1)
24	$6.8\ 10^2 \pm 8.8\ 10^1$ (2.8 ± 0.1)	$4.1\ 10^2 \pm 9.8\ 10^1$ (2.6 ± 0.1)
48	$6.2\ 10^2 \pm 4.4\ 10^1$ (2.8 ± 0.1)	$1.6\ 10^2 \pm 8.4\ 10^1$ (2.2 ± 0.1)

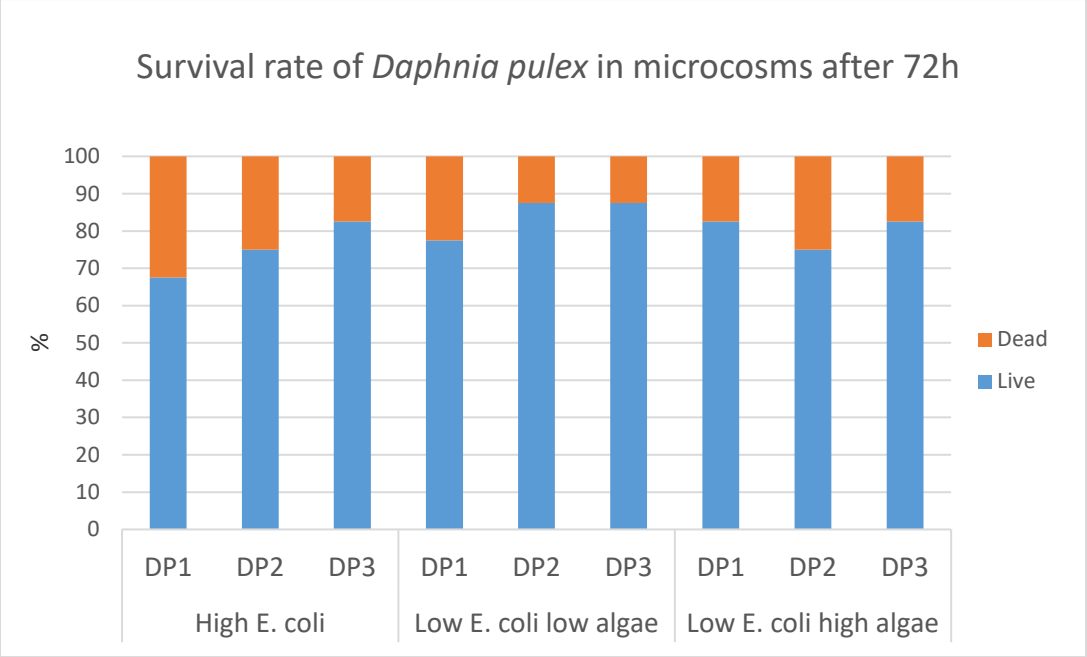

Figure A. 1 : Survival rate of Daphnia pulex in microcosms after 72h (Synthetic water experiment).

2.2 Lake water results

Table A. 6 : Temporal evolution of *E. coli* upon exposure to *D. pulex*. Results are expressed as average ± standard deviation (n=3). Results are expressed in CFU.mL^{-1} (log CFU.mL^{-1}).

Time (hours)	Condition 0 Control microcosms (ADaM)	Condition 1 Control microcosms (R.L.W*)
0	$9.6\ 10^2 \pm 1.1\ 10^2$ (3 ± 0.1)	$1\ 10^3 \pm 2.9\ 10^1$ (3 ± 0.1)
24	$9.6\ 10^2 \pm 6.1\ 10^1$ (3 ± 0.1)	$7.7\ 10^2 \pm 1.3\ 10^2$ (1.3 ± 0.1)
48	$9.2\ 10^2 \pm 5.7\ 10^1$ (3 ± 0.1)	$1.9\ 10^2 \pm 1.2\ 10^2$ (2.2 ± 0.1)

*Raw Lake Water.

Time (hours)	Condition 2 Control microcosms (F.L.W*)	Condition 3 *Daphnia* microcosms 10ind/ml (F.L.W)
0	$9.6\ 10^2 \pm 1.1\ 10^2$ (3 ± 0.1)	$1\ 10^3 \pm 2.9\ 10^1$ (3 ± 0.1)
24	$9.6\ 10^2 \pm 6.1\ 10^1$ (3 ± 0.1)	$7.7\ 10^2 \pm 1.3\ 10^2$ (1.3 ± 0.1)
48	$9.2\ 10^2 \pm 5.7\ 10^1$ (3 ± 0.1)	$1.9\ 10^2 \pm 1.2\ 10^2$ (2.2 ± 0.1)

*Filtered Lake Water on a 53µm mesh size net.

Time (hours)	Condition 4 *Daphnia* microcosms 40ind/ml (F.L.W)	Condition 5 *Daphnia* microcosms 80ind/ml (F.L.W)
0	$9.7\ 10^2 \pm 1.7\ 10^2$ (3 ± 0.1)	$9.3\ 10^2 \pm 2.7\ 10^2$ (3 ± 0.1)
24	$4.4\ 10^2 \pm 8.5\ 10^1$ (2.6 ± 0.1)	$2.9\ 10^2 \pm 2.6\ 10^1$ (2.5 ± 0.1)
48	$7.6\ 10^1 \pm 5.1\ 10$ (1.9 ± 0.1)	$3.8\ 10^1 \pm 1.6\ 10^1$ (1.6 ± 0.1)

2.3 *Daphnia spp* and rotifers results

2.3.1 First assay

Table A. 7 : Temporal evolution of *E. coli* upon exposure to *D. pulex*. *D. magna* (clones 1 and 2). Results are shown as average ± standard deviation (n=3) and expressed in $CFU.mL^{-1}$ (log $CFU.mL^{-1}$).

Condition 1	
Time (hours)	Control
0	$7.4\ 10^2 \pm 9.1\ 10^1$ (2.9 ± 0.1)
24	$2\ 10^2 \pm 2.5\ 10^1$ (2.3 ± 0.1)
48	$2.7\ 10^1 \pm 5.2\ 10$ (1.4 ± 0.1)

Condition 2	
Time (hours)	*D. pulex*
0	$6.3\ 10^2 \pm 6.3\ 10^1$ (2.8 ± 0.1)
24	$4.3\ 10^1 \pm 5.8\ 10$ (1.6 ± 0.1)
48	$3.2\ 10 \pm 3.8\ 10^{-1}$ (5 ± 0.1)

Condition 3	
Time (hours)	*D. magna* clone 1
0	$6.5\ 10^2 \pm 4.8\ 10^1$ (2.8 ± 0.1)
24	$2.7\ 10^1 \pm 1.4\ 10^1$ (1.4 ± 0.1)
48	$5.2\ 10 \pm 1.7\ 10^{-1}$ (7.2 ± - 0.1)

Condition 4	
Time (hours)	*D. magna* clone 2
0	$7.1\ 10^2 \pm 9.6\ 10^{-2}$ (2.9 ± 0.1)
24	$3.8\ 10^1 \pm 7.6\ 10^{-2}$ (1.6 ± 0.1)
48	$1.4\ 10 \pm 3.6\ 10^{-1}$ (7.2 ± - 0.1)

2.3.2 Second assay

Table A. 8 : Temporal evolution of *E. coli* upon exposure to *D. pulex*. *D. magna* and *Brachionus calyciflorus*. Results are expressed as average ± standard deviation (n=3). Results are expressed in $CFU.mL^{-1}$ (log $CFU.mL^{-1}$).

Condition 1	
Time (hours)	Control
0	$4.4\ 10^2 \pm 1.3\ 10^2$ (2.6 ± 0.1)
24	$4.2\ 10^2 \pm 4.1\ 10^1$ (2.6 ± 0.1)
48	$3.2\ 10^2 \pm 1.8\ 10^1$ (2.5 ± 0.1)

Condition 2	
Time (hours)	*D. magna* clone 2
0	$4.2\ 10^2 \pm 7.4\ 10^1$ (2.6 ± 0.1)
24	$3\ 10^1 \pm 4.3\ 10$ (1.5 ± 0.1)
48	$3.2\ 10 \pm 1.6\ 10$ $(- 4.6 \pm 0.1)$

Condition 3	
Time (hours)	*D. pulex*
0	$4.8\ 10^2 \pm 7.2\ 10^1$ (2.7 ± 0.1)
24	$2.4\ 10^2 \pm 1.7\ 10^1$ (2.4 ± 0.1)
48	$1.1\ 10^2 \pm 4.4\ 10^1$ (2 ± 0.1)

Condition 4	
Time (hours)	*B calyciflorus*
0	$4.9\ 10^2 \pm 6.6\ 10^1$ (4.9 ± 0.1)
24	$3.8\ 10^2 \pm 2\ 10^1$ (2.6 ± 0.1)
48	$2.4\ 10^2 \pm 2.1\ 10^1$ (2.4 ± 0.1)

Table A. 9 : Physico-chemical measurements for all conditions, during the whole experiment (Second assay), measurements of PH, Oxygen (mg/L), Temperature (°C), and Turbidity (UNT).

Control Microcosm					
Time [hours]	Average of PH	Average of O_2 (mg / L)	Average of Temperature (°C)	Average of Turbidity (NTU)	Average of Conductivity (µS/cm)
0	7	10	12	32	175
24	7	9	20	14	179
48	7	7	20	11	182

Daphnia magna Microcosm					
Time [hours]	Average of PH	Average of O_2 (mg / L)	Average of Temperature (°C)	Average of Turbidity (NTU)	Average of Conductivity (µS/cm)
0	7	11	12	30	190
24	7	9	20	8	192
48	8	7	20	10	195

Daphnia pulex Microcosm					
Time [hours]	Average of PH	Average of O_2 (mg / L)	Average of Temperature (°C)	Average of Turbidity (NTU)	Average of Conductivity (µS/cm)
0	7	11	11	31	187
24	7	9	20	10	191
48	8	7	20	7	194

Rotifer *Brachionus calyciflorus* Microcosm					
Time [hours]	Average of PH	Average of O_2 (mg / L)	Average of Temperature (°C)	Average of Turbidity (NTU)	Average of Conductivity (µS/cm)
0	7	29	13	29	180
24	7	9	20	14	180
48	7	7	20	10	181

2.4 Impact of natural zooplankton communities on the decay rates of *E. coli* in lake water results

Table A. 10 : Zooplankton community composition of Missisquoi Bay water used for assessing the grazing pressure on *E. coli* using the dilution method (*na = not available).

	10-jun	24-jun	7-jul	29-jul	13-aug	23-sept	21-oct
Herbivorous rotifers	29.6	862	737	139	220	1438	700
Carnivorous rotifers	40.8	545	0	1.1	26.7	0	153
Total rotifers	71	1407	737	140	247	1438	853
Small cladocerans	0.8	6.7	1.7	7.8	6.7	750	45
Large cladocerans	0.4	0	0	0	0.8	0	0
Predatory cladocerans	0	0	0	0	0	0	0
Total cladocerans	1.2	6.7	1.7	7.8	7.5	750	45
Calanoid copepodites	0	0	0	0	0	25.0	15.0
Cyclopoids copepodites	1.4	1.7	5.0	6.7	1.7	37.5	12.5
Herbivorous calanoids	0	0	1.7	1.1	0	0	2.5
Omnivorous cyclopoids	0.2	2	0	0	0	0	2.5
Carnivorous calanoids	0	0	0	0	0	0	0
Total copepods	1.6	3.7	6.7	7.8	1.7	62.5	32.5
Nauplii	19.2	48.3	36.7	21.7	17.5	45.8	415
Predatory invertebrates	0	0	0	0	0	0.1	0
HNF	na*	na*	1229	770	218	1024	915
Ciliates	2356	3013	6209	3142	4491	2107	1996

Table A. 11 : Physico-chemical and nutriment composition of Missisquoi bay water used for assessing the grazing pressure on *E. coli* using the dilution method.

	10-jun	24-jun	7-jul	29-jul	13-aug	23-sept	21-oct
Temperature (°C)	20.1	21.5	22.2	25.4	22.0	20.7	8.5
pH	7.2	7.7	7.9	8.1	8.1	8.1	7.7
Conductivity ($\mu S.cm^{-1}$)	104.5	128.0	136.5	145.8	141.5	142.0	143.8
DO ($mg.L^{-1}$)	9.2	8.7	8.9	8.6	8.3	9.2	11.3
Turbidity (NTU)	3.8	4.3	9.1	1.9	5.5	8.7	5.6
TSS ($mg.L^{-1}$)	-	-	-	-	-	-	-
DOC ($mg.L^{-1}$)	4.4	5.7	5.7	5.9	6.2	7.0	6.3
TOC ($mg.L^{-1}$)	4.8	5.8	5.8	6.0	6.4	7.5	6.7
NH_3-N ($mgN.L^{-1}$)	0.06	<.05	<.05	<.05	<.05	<.05	<.05
NO2+3-N ($mgN.L^{-1}$)	0.45	0.47	0.37	0.20	0.16	0.09	0.13
TKN-N ($mgN.L^{-1}$)	-	-	2.04	-	3.31	5.40	4.25
P.total ($mgP.L^{-1}$)	-	-	0.09	-	0.11	0.13	0.12
Ortho-P ($mgP.L^{-1}$)	0.02	<.02	-	<.02	<.02	<.02	0.03

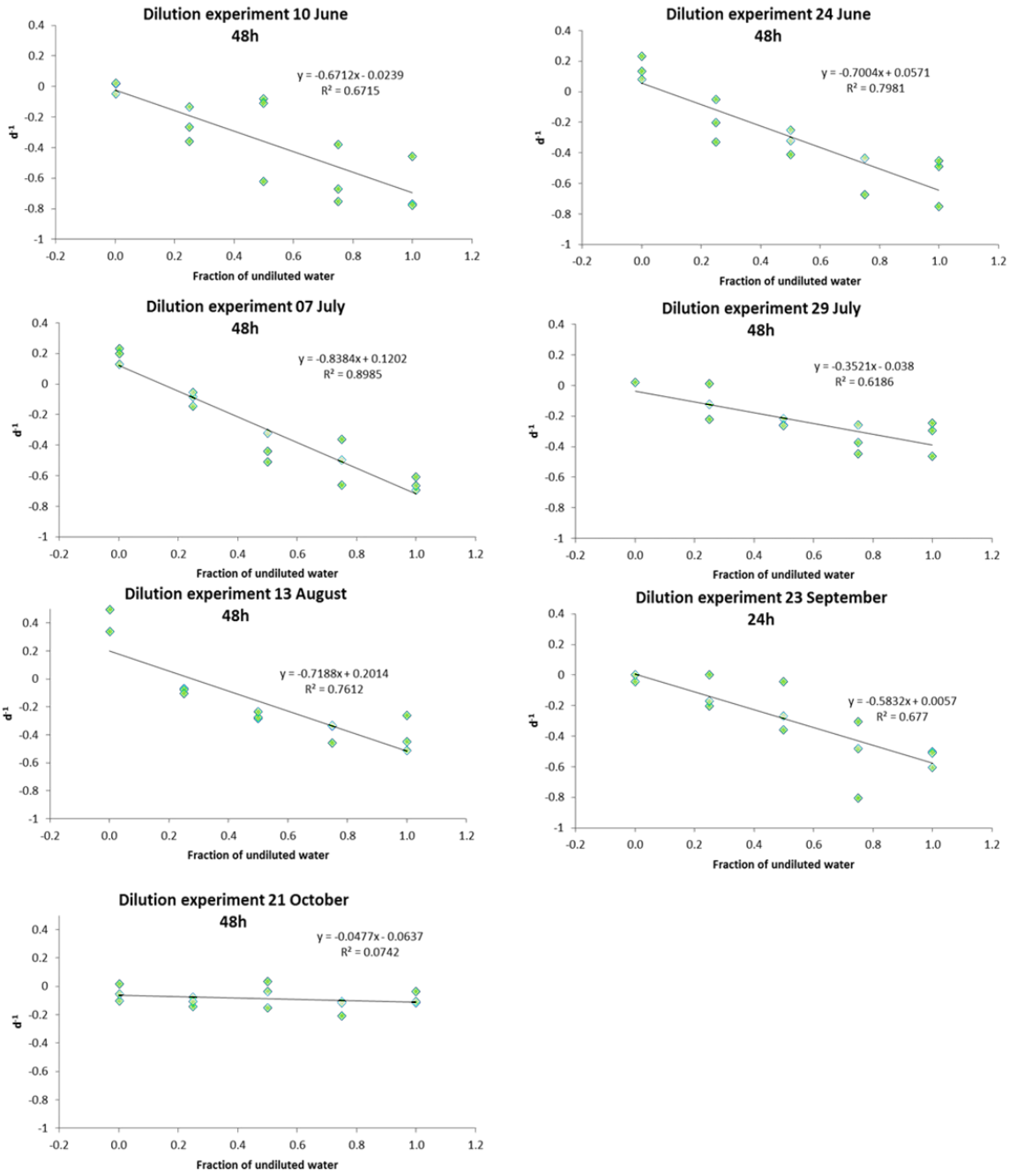

Figure A. 2 : Zooplankton community grazing rates (d^{-1}) as determined by the dilution method for the 7 sampling occasions (June to October 2015).

3. The location of collected Moina sp, Saint-Hyacinthe wastewater treatment plant.

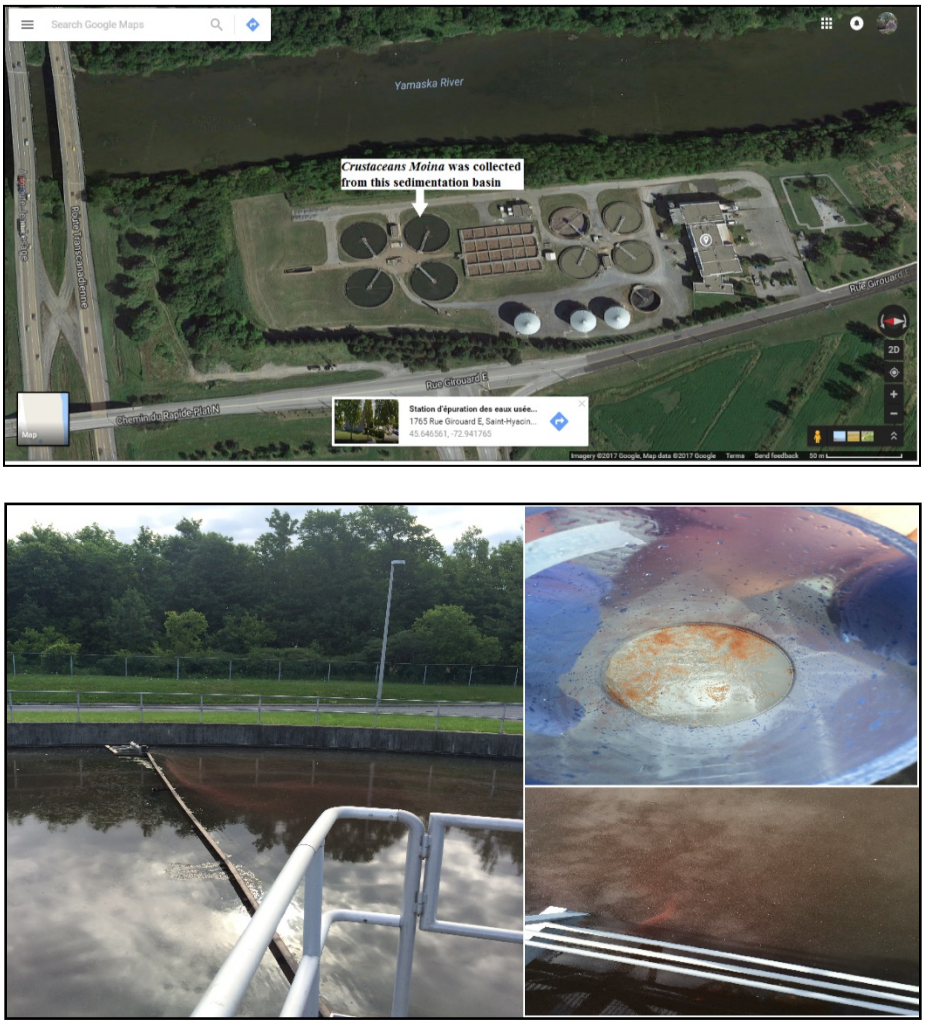

Figure A. 3 : Location of Saint-Hyacinthe wastewater treatment plant (google map), and collecting the *Moina sp* from the sedimentation basins by using zooplankton net (53 µm).